Parasites
ELITZA S. THEEL1 and BOBBI S. PRITT1
1Mayo Clinic, Rochester, MN 55905

ABSTRACT Parasites are an important cause of human disease worldwide. The clinical severity and outcome of parasitic disease is often dependent on the immune status of the host. Specific parasitic diseases discussed in this chapter are amebiasis, giardiasis, cryptosporidiosis, cyclosporiasis, cystoisosporiasis, microsporidiosis, granulomatous amebic encephalitis, toxoplasmosis, leishmaniasis, Chagas disease, malaria, babesiosis, strongyloidiasis, and scabies.

INTRODUCTION Parasites are an important cause of human disease worldwide. Malaria alone was responsible for 584,000 estimated deaths in 2013, while millions of others died from Chagas disease, African trypanosomiasis, strongyloidiasis, amebiasis, leishmaniasis, ascariasis and schistosomiasis (1). Eleven parasitic infections have been identified by the World Health Organization (WHO) as neglected tropical diseases because they threaten the health of millions of individuals and disproportionately impact impoverished individuals (2). The Centers for Disease Control and Prevention (CDC) has also identified five parasitic infections that have significant public health implications for individuals living in the United States, demonstrating that parasitic infections are not limited to the tropical regions of the world.

The clinical severity and outcome of parasitic disease is often dependent on the immune status of the host (Table 1) (3). Humans protect themselves from parasites using a variety of defenses, including nonspecific immunity (e.g., mucosal barriers, pH, temperature), innate mechanisms (e.g., complement, toll-like receptors) and adaptive immune mechanisms (e.g., pathogen-specific humoral and cell-mediated responses). In general, helminths elicit a protective helper T-lymphocyte type 2 (Th2) host response with production of IgE, eosinophilia and mastocytosis (4, 5), while protozoa elicit a humoral and/or cellular immune response that is not generally associated with eosinophilia. Conditions that compromise host immunity, thus making individuals more susceptible to parasitic infections and associated disease complications, include primary or acquired immunodeficiencies, receipt of immunosuppressive therapies, malnutrition, and asplenia. Neonates, the elderly and those with chronic disease may also be at increased risk of acquiring or suffering severe consequences from parasitic infections. This chapter will focus on those diseases that pose the greatest risk to patients whose immune systems are compromised.

INTESTINAL PROTOZOA The enteric protozoan parasites are the most common parasitic diseases worldwide and often cause severe, prolonged symptoms in patients with deficient or impaired cellular immunity, including patients with AIDS and those receiving immunosuppressive therapies for solid organ or stem cell transplants (6–9). When compared to immunocompetent individuals, immunocompromised patients are more likely to acquire infection after exposure, generally experience more severe and even disseminated disease, and are often unable to clear the infection, even with antiparasitic therapy (6). They may also experience reactivation of previously controlled parasites such as Toxoplasma gondii and...
**TABLE 1** Manifestation of parasitic infections in immunocompetent and immunocompromised hosts

<table>
<thead>
<tr>
<th>Organism (Disease)</th>
<th>Clinical Manifestations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Immunocompetent host</strong></td>
<td><strong>Immunocompromised host</strong></td>
</tr>
<tr>
<td>Babesia species (babesiosis)</td>
<td>Usually asymptomatic parasitemia or self-limited flu-like illness, hemolytic anemia</td>
</tr>
<tr>
<td>Cryptosporidium species (cryptosporidiosis)</td>
<td>Self-limited watery diarrhea</td>
</tr>
<tr>
<td>Cyclospora cayetanensis (cyclosporiasis)</td>
<td>Self-limited watery diarrhea</td>
</tr>
<tr>
<td>Cystoisospora belli (cystoisosporiasis)</td>
<td>Self-limited watery diarrhea</td>
</tr>
<tr>
<td>Entamoeba histolytica (amebiasis)</td>
<td>Most asymptomatic; manifestations include colitis, ameboma, amebic liver abscess and disseminated disease</td>
</tr>
<tr>
<td>Free-living amebae</td>
<td>Amebic keratitis, usually in contact-lens wearers</td>
</tr>
<tr>
<td>Giardia duodenalis (giardiasis)</td>
<td>Most with asymptomatic or mild, self-limited watery diarrhea, malabsorption and flatulence; small percentage experience chronic fatigue, reactive arthritis and allergies</td>
</tr>
<tr>
<td>Leishmania species (leishmaniasis)</td>
<td>Many individuals are asymptomatic; cutaneous and visceral leishmaniasis may occur</td>
</tr>
<tr>
<td>Microsporidia (microsporidiosis)</td>
<td>Self-limited watery diarrhea, ocular microsporidiosis</td>
</tr>
<tr>
<td>Plasmodium species (malaria)</td>
<td>Disease severity is species-dependent, with P. falciparum considered the most severe; high fever, malaise, anemia; rarely respiratory distress and cerebral malaria</td>
</tr>
<tr>
<td>Sarcoptes scabei (scabies)</td>
<td>Asymptomatic to moderate itching</td>
</tr>
<tr>
<td>Strongyloides stercoralis (strongyloidiasis)</td>
<td>Asymptomatic to mild abdominal pain, diarrhea; autoinfective cycle allows for chronic infection; suppressed by immune system</td>
</tr>
<tr>
<td>Toxoplasma gondii (toxoplasmosis)</td>
<td>Asymptomatic or mild, self-limited mononucleosis-like illness; transmission to fetus during primary maternal infection can lead to severe developmental sequelae</td>
</tr>
<tr>
<td>Trypanosoma cruzi (Chagas disease)</td>
<td>Acute, intermediate and chronic stages; cardiomyopathy, megacolon and megaesophagus may arise years after initial infection</td>
</tr>
</tbody>
</table>

*Adapted from reference 311.

**Leishmania spp.** Of the intestinal protozoa that infect humans, *Entamoeba histolytica*, *Giardia duodenalis*, *Cryptosporidium spp.*, *Cyclospora cayetanensis*, *Cystoisospora belli* and the microsporidia (now categorized as fungi) produce the greatest burden of disease morbidity and mortality in the immunocompromised population (6, 7, 9). HIV-infected individuals and other patients with defects in cell-mediated (T-cell) immunity are at particular risk of acquiring infections with pathogenic enteric protozoa; therefore, testing for these parasites should be considered in immunocompromised patients with prolonged or severe watery diarrhea and an appropriate exposure history (6, 10).

When possible, reversal of the underlying immunocompromising condition is an important component of treating these infections (9). Additionally, immunocompromised patients should be counseled on the risks of ingesting food or water that is potentially contaminated with fecal material (e.g., when traveling to endemic countries), the importance of handwashing before eating and the risks of unprotected anal sex.

**Entamoeba histolytica (Amebiasis)**

**Introduction**

*Entamoeba histolytica* is a protozoan parasite with a global distribution. It falls within the phylum Amoebozoa, along with several other *Entamoeba* spp. that are capable of infecting humans. *E. histolytica* is considered to be the only definitive human pathogen in the genus (11).

**Epidemiology**

Our ability to estimate the prevalence of *E. histolytica* infection has long been hindered by our inability to dif-
ferentiate E. histolytica from E. dispar, E. moshkovskii, and E. bangladeshi using light microscopy. Prior to the discovery of these other species, all similar-appearing Entamoeba were considered to be E. histolytica, thus greatly overestimating the prevalence of infection. Studies using methods that are capable of differentiating the different Entamoeba species now show that E. dispar is up to ten times more common than E. histolytica in asymptomatic patients from endemic regions (12–15). Although significant regional variability exists throughout the world, there are an estimated 34 to 50 million symptomatic cases each year with 40,000 to 110,000 deaths (16). Infection is most common in settings of poor sanitation and lack of adequate sewage and water treatment systems. Infection with E. histolytica is also more commonly reported in men that have sex with men (MSM) (10, 17). In resource-rich countries such as the United States, amebiasis is primarily seen in immigrants and travelers from endemic countries (18). Despite the high prevalence of infection in some regions of the world, amebiasis does not appear to be a major source of morbidity and mortality in immunocompromised patients; however, some immunocompromised patients may be at increased risk of infection and/or symptomatic disease. Refer to Clinical presentation below for further details.

Life cycle and transmission to humans
Infection with Entamoeba histolytica is acquired primarily through the fecal–oral route via ingestion of cysts in contaminated food or water. Following ingestion, the organisms excyst in the small intestine, releasing trophozoites, which colonize the large intestine (6). Trophozoites feed on intestinal bacteria and multiply by binary fission. Ultimately, most trophozoites convert to cysts, which are shed into the environment through the stool and are immediately infectious. In a small percentage of cases, the trophozoites invade into the bowel wall and cause symptomatic disease. Rarely, they may enter portal circulation and disseminate to the liver and other organs (18, 19).

Pathophysiology
Although the signals that mediate transition from asymptomatic colonization to symptomatic invasion are not well understood, several parasite virulence factors have been recognized that play a role in invasion and destruction of host tissue (20). E. histolytica trophozoites first gain access to the intestinal mucosa by degrading the protective colonic mucin using secreted proteases and glycosidases. Adherence to the intestinal epithelium is then mediated by galactose/N-acetylgalactosamine lectin and other membrane proteins and results in epithelial cell death via amoebapore activity and induction of apoptosis (20, 21). Exposed epithelial cells recruit neutrophils and macrophages through release of potent chemokines such as IL-8, which when activated by exposure to tumor necrosis factor-α (TNF-α), interferon gamma (IFN-γ) or lipopolysaccharide, have potent amebicidal activity. Of note, amebic trophozoites are highly effective at lysing and phagocytosing neutrophils and modulating macrophage responses. Destruction of neutrophils results in release of inflammatory mediators that contribute to ongoing host cell damage (20). During dissemination, trophozoites can resist complement-mediated damage using specific parasite surface molecules, such as the galactose/N-acetylgalactosamine-specific lectin, peroxidase, proteases, and lipopolysaccharide (20, 22). Host factors that provide protection against E. histolytica include IgA antibodies against the galactose/N-acetylgalactosamine lectin and production of IFN-γ. Other host factors such as leptin signaling and human leukocyte antigen polymorphisms are also thought to play an important role in determining the outcome of infection (20). Since production of proinflammatory cytokines such as IFN-γ and TNF-α facilitate amebic destruction, individuals with impaired cellular immunity may be at higher risk of symptomatic disease and complications, but this has yet to be definitively proven by clinical studies.

Clinical presentation
Infection with E. histolytica, regardless of clinical presentation, is referred to as amebiasis (16). The majority (90%) of patients with E. histolytica infection are asymptomatic. These individuals may have a low or undetectable antibody titer but can pass infective cysts in their stool and serve as an important reservoir for E. histolytica. Only 10% to 20% of infected individuals will develop symptomatic disease, and this usually manifests as colitis with abdominal pain and mucoid, watery or bloody diarrhea (18). Other forms of intestinal disease include acute fulminating colitis, appendicitis, toxic megacolon, and formation of a tumor-like inflammatory mass in the colon called an ameboma (18). Invasion by E. histolytica trophozoites is impeded by the outer muscular layer of the bowel wall, thus favoring lateral invasion into the submucosa. The resultant ulcers have a base, which is broader than the apex, thus giving them the characteristic “flask shape” (18). Ulcers are found most commonly in the cecum, ascending colon and appendix but may also be found in the rectosigmoid region.
of the colon (Fig. 1). Invasion of the bowel wall brings *E. histolytica* trophozoites into direct contact with the portal blood supply and may result in extraintestinal dissemination and abscess formation in a variety of organs, most commonly the liver. Amebic liver abscess (ALA) is associated with right upper-quadrant pain, weight loss, and fever and occurs 3 to 20 times more often in men ages 18 to 50 than in other populations (18). Up to 75% of patients with ALA do not have intestinal symptoms, and thus, diagnosis requires a high degree of clinical suspicion and appropriate testing (23).

As described above, the outcome of infection is dependent on the interaction between the host immune response and parasite virulence factors. Patients at increased risk for severe disease include infants, the elderly, immunocompromised patients, pregnant women, and those with underlying conditions such as malignancy, alcoholism, malnutrition and diabetes (18, 24, 25). Interestingly, it is unclear whether HIV infection predisposes individuals to severe disease. A study of 203 Mexican patients with HIV/AIDS and 140 of their HIV-negative close contacts found no significant difference in the specific detection of *E. histolytica* between the two groups when using PCR testing of stool specimens (26). In addition, no significant association between presence of *E. histolytica* infection and CD4+ T lymphocyte cell counts was documented, although their sample size was relatively small. None of the individuals with *E. histolytica* infection in this study had symptoms of amebiasis in the 12 months preceding the study. The authors’ conclusion from this study was that “infection by HIV is not a risk factor for amebiasis” (26).

Similarly, in an Australian study of MSM with diarrhea, *E. histolytica* infection was more prevalent in HIV-negative rather than HIV-positive patients, as was infection with *E. dispar* or *E. moshkovskii* (10). In contrast, studies from China and Taiwan found that HIV-infected individuals had a higher rate of *E. histolytica* antibodies than patients without HIV infection (27, 28), and seropositivity has been associated with invasive disease. Similarly, ALA has been described as an emerging disease in HIV-positive patients (29, 30). Further studies examining specific populations (e.g. MSM, intravenous drug users, female sex workers) and sexual practices (e.g., oral–anal sex) is necessary to tease apart the potential risk factors for amebiasis and symptomatic disease in HIV-infected individuals.

### Diagnosis

The preferred diagnostic modality for amebiasis varies with the type of disease. While detection of the parasite, its antigens or DNA in stool is commonly performed for identification of intestinal disease, detection of circulating IgM or IgG antibodies reacting to *E. histolytica* antigens is the preferred method for detection of extraintestinal disease. It is important to mention that not all techniques are capable of differentiating *E. histolytica* from closely related parasites, but species-level identification (discussed below) is recommended for guiding appropriate treatment (16).

### Direct detection by microscopy

Characteristic *Entamoeba* cysts and trophozoites can be detected by examining direct (fresh) or preserved stool specimens (Fig. 1), (31, 32). Trophozoite size ranges from 10 to 60 μm in length (usually 15 to 20 μm), and it has a single nucleus with a characteristic “Entamoeba”-type chromatin pattern (central dot and peripheral condensed rim of chromatin). The cytoplasm is finely granular and may contain engulfed bacteria. Rarely, in cases of invasive amebiasis, engulfed red blood cells (RBCs) may be observed. Trophozoites may demonstrate “directional” motility when examining direct, unfixed stool specimens; however, this is an insensitive method for detection. Mature cysts measure 10 to 20 μm in diameter and have four nuclei. Rod-shaped cytoplasmic chromatoid bodies with rounded ends are commonly observed. It is important to note that the trophozoites of *E. histolytica* are morphologically identical to those of *E. dispar*, *E. moshkovskii*, and *E. bangladeshi*, and therefore a definitive species-level identification cannot be rendered based on morphology alone. The only exception to this rule is when RBCs are observed within the trophozoite cytoplasm; in that case, a presumptive diagnosis of *E. histolytica* may be rendered (16). As with many other protozoal infections, release of *E. histolytica* trophozoites or cysts into human feces is sporadic, thus necessitating collection of three or more specimens to detect the majority (85% to 95%) of cases (32).

Less commonly, histopathologic examination of intestinal biopsies may be useful for demonstrating the invading trophozoites (usually observed in the submucosa at the edge of the “flask-shaped” ulcer) and for ruling out noninfectious conditions. Microscopy is an insensitive method (<10% sensitive) for detection of extraintestinal infections when examining aspirates or biopsies. In particular, material aspirated from amebic liver abscesses is composed primarily of degenerated organisms and necrotic hepatocytes (said to resemble anchovy paste), and recognizable trophozoites are rarely present. The majority of cases of ALA do not have a concomitant
**FIGURE 1** *Entamoeba histolytica*. (A) Resected segment of colon showing multiple ulcers with focal perforation due to amebiasis. (B) Amebic liver abscess with vial of aspirated abscess material demonstrating the characteristic "anchovy paste" appearance (Gross photos courtesy of Dr. Mae Melvin and Dr. E. West, CDC Public Health Image Library). (C) *E. histolytica*/*E. dispar* trophozoite (left) and cyst (right) (modified trichrome, 1000×). Reproduced with permission from Pritt BS. *Parasitology Benchtop Reference Guide*. Northfield, IL: College of American Pathologists, 2014. If ingested RBCs are seen within the cytoplasm of the trophozoite, then a presumptive diagnosis of *E. histolytica* can be made. Otherwise, the trophozoites and cysts of *E. histolytica* and *E. dispar* are indistinguishable. Note that two of the four nuclei are visible within the cyst as well as a prominent chromatoid body.
intestinal infection, thus rendering stool examination noncontributory to diagnosis in this setting. Therefore, serology is the method of choice for detection of extra-intestinal disease.

**Antigen detection**

Several enzyme-linked immunosorbent assay (ELISA) and immunochromatographic assay-based kits are commercially available and offer similar sensitivity for *E. histolytica* detection compared to standard microscopy. Some (but not all) kits can provide definitive species-level identification of *E. histolytica* and may be used as a supplemental tool when morphologically consistent cysts or trophozoites are identified by microscopy. Some assays can also be used for testing liver abscess material, although the reported sensitivity is low (32).

**Molecular methods**

Molecular methods such as PCR offer the highest sensitivity of all laboratory diagnostic methods for detection of *E. histolytica* in stool or abscess material, and most have the ability of providing species-level identification (32). Historically, the use of these assays was limited primarily to the research or reference lab setting. However, the recent introduction of commercial multiplex nucleic acid amplification tests (NAATs) for detection of multiple enteric pathogens, including *E. histolytica*, is expected to greatly expand the availability of molecular tests in the routine diagnostic laboratory in resource-rich settings (33). While performance data specifically for the *E. histolytica* target of these multiplex assays remains limited, preliminary findings suggest high sensitivity, ranging from 83% to 99% (33). Unfortunately, most molecular methods are impractical for use in many endemic settings due to limitations with personnel, equipment, and facility requirements.

**Serology**

As mentioned previously, serological tests for detection of *E. histolytica* infection are the preferred method for detecting extraintestinal disease in the absence of intestinal disease, with reported sensitivities approaching 100% (23, 32). They may also detect invasive intestinal disease when the parasites have interacted sufficiently with the host’s immune system to generate a detectable serologic response. Unfortunately, serologic methods cannot differentiate between past and current disease and therefore have limited clinical utility in endemic settings. Additionally, not all serologic assays can differentiate antibodies to *E. histolytica* from antibodies to the other morphologically identical species. Many different assays have been described, including those using indirect hemagglutination, immunodiffusion, indirect immunofluorescent assay (IFA), latex agglutination immunoelectrophoresis, and ELISA, and several of these are commercially available (32).

**Culture**

Culture is a traditional method that has been used for the isolation of *Entamoeba* spp. for over 80 years. It may be performed using xenic or axenic systems but requires significant technical expertise and is rarely used in the clinical laboratory today. It remains a useful technique in the research laboratory and provides sufficient quantity of organisms for downstream isoenzyme and genetic analysis.

**Treatment**

The WHO and Pan American Health Organization (PAHO) recommend treating all cases of proven *E. histolytica* infection, regardless of symptoms, given the small but substantial risk of invasive disease and transmission to others (16). If only *E. dispar* is detected (or other nonpathogenic amebae), treatment is not indicated; therefore, the use of diagnostic methods capable of differentiating *E. histolytica* from other *Entamoeba* spp. is important for patient management. Asymptomatic infection should be treated with a luminal amebicide such as iodoquinol, paromomycin or diloxanide furoate to eradicate the intestinal infection and prevent subsequent tissue invasion (34). In contrast, invasive intestinal disease or extraintestinal disease should be treated with tissue amebicides, such as metronidazole, and followed by therapy with a luminal agent to eradicate any intestinal infection. Drainage of amebic liver abscesses is not generally recommended due to the risk of rupture or content spillage (11).

**Control**

Efforts to control and prevent amebiasis largely focus on improvements in hygiene and sanitation. Immunocompromised patients should receive counseling as indicated above.

**Giardia duodenalis** (Giardiasis)

**Introduction**

*Giardia duodenalis*, also known as *G. intestinalis* and *G. lamblia*, is a flagellated intestinal protozoan in the phylum Metamonada that causes disease, referred to as giardiasis, worldwide (35). In addition to humans, it is capable of parasitizing numerous other animals including mammals, reptiles and birds (6).
Epidemiology
Giardiasis has two main epidemiologic patterns. Infection is endemic in regions with warm climates and poor sanitation, where nearly one-third of individuals will be infected at some point in their lives (36). In these settings, children are commonly infected and shed large numbers of cysts in their stool (31). Infection is transmitted primarily by person-to-person contact, and clinical immunity develops over time with continued exposure. In contrast, giardiasis causes sporadic epidemics in resource-rich settings with good sanitation practices and is usually transmitted via consumption of contaminated food or untreated ground or surface water (37). Without preexisting immunity, symptomatic disease occurs in both children and adults. *G. duodenalis* is the most commonly reported intestinal parasite in the United States, with >15,000 cases occurring annually (36, 37). Individuals at greatest risk of acquiring infection are hikers and campers who drink untreated surface water, children, caregivers in child-care settings, travelers to regions where giardiasis is endemic, individuals who have contact with infected animals, and MSM.

There are few studies describing giardiasis in immunocompromised patients, although data suggest that patients with hypogammaglobulinemia, X-linked infantile congenital hypogammaglobulinemia, or common variable (late-onset)-acquired hypogammaglobulinemia are at increased risk of infection and symptomatic disease (6). Children with lymphoma, protein-calorie malnourishment or those who are receiving corticosteroids for nephrotic syndrome have also been found to be at increased risk of infection (38). In contrast, children with severe T-cell deficiencies are not more predisposed to infection than immunocompetent children. Patients with AIDS also do not appear to be at higher risk of infection than immunocompetent individuals (39).

Life cycle and transmission to humans
*Giardia* is transmitted via ingestion of infective cysts in contaminated food or water or via person-to-person contact, including oral/anal sexual practices. Less commonly, infection can also be transmitted by animal-to-person contact (37). The environmentally resistant cysts are infectious immediately following excretion and can withstand cold water and moderate levels of chlorine. Infected individuals are estimated to shed >100 cysts in their stool daily, while ingestion of as few as 10 cysts can result in infection (37). Excystation occurs in the small intestine, releasing the active trophozoite form which multiplies by binary fission in the lumen of the bowel. Trophozoites then undergo encystation as they are carried into the large intestine, resulting in shedding of cysts, and occasionally trophozoites, in the feces (36).

Pathophysiology
Trophozoites adhere to the small-intestinal mucosa via a ventral sucking disk, resulting in enterocyte microvillus damage and malabsorption (40). Crypt hyperplasia, villus atrophy, and increased numbers of neutrophils may also be observed, although normal histology has been observed in small intestinal biopsies from patients with symptomatic giardiasis (40). The parasite is able to undergo continuous surface antigen variation, which may play a role in immune avoidance. Interestingly, increased jejunal antigen uptake and delayed recruitment of mast cells has been observed during the acute stage of infection, which may be associated with the increased incidence of allergies and hypersensitivity reactions in patients with giardiasis (35). The mechanisms behind acquired immunity are poorly understood and may involve both humoral and cell-mediated immune mechanisms. At this time, no clear correlation has been established between development of antibodies against specific *Giardia* antigens and protection against infection.

Clinical presentation
Most infected individuals (>50%) are asymptomatic or have only mild, self-limited disease. When present, symptoms usually include diarrhea, bloating, malabsorption, abdominal cramps, and flatulence (36). Following acute infection, some individuals progress to subacute or chronic infection and may have long-standing symptoms such as intermittent diarrhea and abdominal discomfort. Chronic fatigue, reactive arthritis, and allergies have also been described (37).

As mentioned above, certain immunocompromised states have been associated with increased disease severity. However, the infection does not appear to be more severe in patients with HIV infection, despite the elevated risk of symptomatic infection associated with reduction of CD4+ counts (6).

Diagnostic
Direct detection by microscopy
Giardiasis is commonly diagnosed by finding characteristic cysts and trophozoites in fecal specimens (Fig. 2). Less commonly, duodenal fluids (e.g., aspirates or string test) or biopsies may be examined. Trophozoites have a classic “pear” or “kite” shape and measure 10 to 20 μm in length (usually 12 to 15 μm), while cysts are oval, have four nuclei, and measure 8 to 19 μm (usually 11 to 12 μm) (35). Trophozoites may demonstrate “falling
“leaf” motility when examining direct, unfixed stool specimens, although this is an insensitive method for detection. Cysts predominate in formed stool, whereas both trophozoites and cysts are commonly seen in diarrheic specimens.

Like other protozoa, *Giardia* cysts and trophozoites are intermittently shed in the stool, thus requiring examination of multiple specimens for maximum sensitivity. Even with the use of concentration techniques, the variability of distribution in fecal specimens can make detection of this parasite challenging when using routine microscopic examination. Therefore, fecal immunoassays, which offer greater sensitivity, are recommended for routine diagnosis (36). Less commonly, histopathologic examination of small-intestinal biopsies may be useful for demonstrating the extracellular trophozoites and for ruling out noninfectious conditions such as celiac disease.

**Antigen detection**

Many antigen detection methods using enzyme immunoassays or direct fluorescence assays are commercially available. These assays generally demonstrate improved sensitivity over routine microscopic stool examination and have high analytical specificity (35). They are therefore useful for testing patients with diarrhea for whom helminth infection is not suspected (e.g., patients who have not travelled outside of the United States). Cartridge-based lateral-flow immunochromatographic assays for *Giardia* antigen detection are also commercially available but are generally less sensitive and specific than other antigen detection methods and should not take the place of routine stool parasite testing (41).

**Molecular methods**

Until recently, NAATs for *Giardia* detection were limited to use in research and public health settings. However, the recent introduction of commercial multiplex NAATs for intestinal parasites including *Giardia* will likely increase the use of these technologies in routine diagnostic microbiology laboratories (33). Similar to molecular testing for *E. histolytica*, there are limited studies evaluating the accuracy of multiplex testing for
Giardia. In general, accuracy is considered high and ranges from 89% to 99%, depending on the selected platform (33).

Serology
Because G. duodenalis does not invade into human tissues, it does not elicit a detectable systemic antibody response. Therefore, serology plays no role in diagnosis of giardiasis (35).

Treatment
Giardiasis is generally treated using members of the nitroimidazole class of drugs such as metronidazole and tinidazole (34). Other options include nitazoxanide, paromomycin, quinacrine and furazolidone. Paromomycin has poor systemic absorbance and therefore may be useful for treating pregnant women. Although standard treatment courses are usually effective, treatment failures have been identified with all of these agents.

Control
Like the other intestinal protozoa, giardiasis is prevented and controlled through institution of improved sanitation and hygiene, and immunocompromised patients should receive counseling regarding these infections, as indicated above (36).

Coccidia
Introduction
The coccidia are obligate intracellular apicomplexan parasites that cause intestinal infection in humans. The most common coccidia to infect humans are Cyclospora cayetanensis and Cystoisospora belli (formerly Isospora belli) (42). Cryptosporidium species are also traditionally grouped with the coccidia, although recent molecular and biologic studies indicate that they are a separate lineage of apicomplexan parasites (43). For simplicity, Cryptosporidium are discussed in this section along with Cyclospora and Cystoisospora because they share an ecologic niche and cause similar clinical symptoms in humans. In general, the risk of developing an infection with one of these enteric parasites is dependent on cell-mediated T-cell function. Not surprisingly, patients with HIV infection or other causes of depressed T-cell activity are specifically at increased risk for acquiring infection and developing severe disease. These patients are also at risk for prolonged carriage and relapses (6).

Life cycle and transmission to humans
Infection with these parasites occurs through the fecal–oral route via ingestion of infectious oocysts in contaminated food or water (31). Following ingestion, the oocysts excyst and release sporozoites which infect epithelial cells of the small intestine, and occasionally the biliary tree. Cryptosporidium may also infect epithelial cells of other tissues including the respiratory tract (31). Parasites undergo both asexual and sexual stages of reproduction within enterocytes. The fusion of male and female gametes results in the formation of oocysts which are then shed in the feces. Oocysts of Cryptosporidium species are infectious at the time of excretion, thereby permitting direct fecal–oral transmission, person-to-person transmission (including through oral–anal sexual contact) and autoinfection (44), whereas oocysts of Cyclospora cayetanensis and Cystoisospora belli must first undergo sporulation in the environment before becoming infectious (6).

Pathophysiology
All three organisms cause disease by invading the small-intestinal epithelium, resulting in villous blunting, crypt hyperplasia and acute and chronic inflammation in the lamina propria. Unlike other protozoa, intestinal and peripheral eosinophilia is also observed with C. belli infection (31).

Epidemiology
Cryptosporidium species have a global distribution and infect a wide range of vertebrate hosts. The most common species to infect humans are Cryptosporidium parvum and Cryptosporidium hominis, although other species, including Cryptosporidium canis, Cryptosporidium felis, Cryptosporidium meleagridis and Cryptosporidium muris can also cause human infections, particularly in immunocompromised hosts (6). In the United States, outbreaks of cryptosporidiosis are commonly associated with exposure to infected animals and contaminated municipal or recreational water sources (44). Person-to-person and autoinfection can also occur. Several studies have found Cryptosporidium to be the most common opportunistic enteric pathogen encountered in HIV-positive patients (6, 8, 10). The largest recorded outbreak of cryptosporidiosis in the United States occurred in Milwaukee in 1993 due to contamination of the municipal water supply (45). Over 400,000 individuals were infected, with at least 104 deaths, primarily among the elderly and patients with AIDS (46, 47).

Unlike Cryptosporidium, Cyclospora and Cystoisospora are parasites of humans only and are found predominantly in developing countries with poor sanitation and hygiene. Outbreaks have been detected throughout
many parts of the world, with recent U.S. outbreaks associated with consumption of fruits and vegetables from Mexico and Central America (48). Cases of cyclosporiasis and cystoisosporiasis in the United States have also been described from persons who traveled to endemic areas such as South America. Immunodeficiency, particularly AIDS, has been shown to increase susceptibility to infection with these parasites (49, 50).

Clinical presentation

In the immunocompetent host, infection with any of these three parasites is commonly associated with self-limited watery diarrhea (5 to 10 bowel movements/day), nausea, vomiting, abdominal pain and low-grade fever. Dehydration is an uncommon complication. In comparison, immunocompromised patients, particularly those with CD4+ lymphocyte counts below 200 cells/μl, are at risk for chronic and more severe disease (6). The risk of autoinfection with cryptosporidiosis may also contribute to prolonged symptoms in immunocompromised hosts.

Specific patient groups at risk for prolonged, severe and potentially life-threatening diarrhea due to cryptosporidiosis include individuals with AIDS, non-Hodgkin’s lymphoma, lymphoproliferative disease, leukemia, malnutrition, and those receiving immunosuppressive drugs for organ transplants or malignancy (6). Serious infections have also been noted in immunocompromised patients undergoing hemodialysis, patients with primary CD4 lymphopenia, and patients with hyper-IgM syndrome. In addition to intestinal disease, organisms may disseminate to the biliary tract, pancreas, and respiratory tract in immunocompromised individuals. In particular, sclerosing cholangitis, pancreatitis and lung involvement are well-known complications of cryptosporidiosis in patients with AIDS (51, 52).

Similarly, severe, prolonged, and even relapsing cyclosporiasis may be seen in patients with AIDS, leading to dehydration and malnutrition. Prolonged symptomatic cyclosporiasis has also been described in patients with protein-energy malnutrition, Hodgkin’s lymphoma, compensated idiopathic hepatic cirrhosis and acute lymphoblastic leukemia. Rarely, disseminated infections may involve the biliary tract (53) and possibly the lungs (54).

Finally, C. belli infection may cause severe, even fulminant and life-threatening, diarrhea and wasting in patients with AIDS (6). Chronic or severe cystoisosporiasis has also been reported in malnourished children, solid organ transplant (SOT) recipients and patients with hematologic malignancies (6). Extraintestinal infection has been reported to involve the biliary tract in patients with AIDS (55) and may also rarely disseminate to lymph nodes, liver and spleen (56). Of the three parasites, cystoisosporiasis is the only one to be associated with peripheral eosinophilia and eosinophils/eosinophil-derived Charcot-Leyden crystals in stool (31). Therefore, this infection should be considered in the differential diagnosis for HIV-positive individuals with persistent nonbloody diarrhea, weight loss, eosinophilia and a history of travel to endemic areas.

Diagnosis

All three parasites are commonly identified in the clinical laboratory by direct visualization of oocysts in stained (e.g., acid-fast stains) and unstained stool preparations; however, antigen detection assays provide improved sensitivity for detection of Cryptosporidium spp. and are currently considered to be the test of choice for this parasite (44). Interestingly, several highly sensitive commercial NAATs for Cryptosporidium and C. cayetanensis have recently become available and will likely expand the number of clinical laboratories using molecular techniques for diagnosis of these parasites (33). Culture and serology are not clinically useful methods.

Direct detection by microscopy

Oocysts of Cryptosporidium spp., C. cayetanensis, and C. belli can be detected in stool using wet preparations, but they are transparent and may be easily overlooked. Detection is enhanced by use of modified acid-fast stains, which stain the oocysts a red-pink color (Fig. 3, 4, and 5). Not all oocysts stain evenly, and some appear as glassy unstained “ghost” cells, a particularly common phenomenon for C. cayetanensis. The oocysts of the different parasites are differentiated by their size and shape: Cryptosporidium oocysts are round and measure 3 to 6 μm in diameter, C. cayetanensis oocysts are round and measure 7 to 10 μm in diameter and C. belli oocysts are ellipsoidal and measure 2.5 to 30 μm at their greatest dimension (31).

Cyclospora belli oocysts also autofluoresce using UV epifluorescence, thus making this a useful screening tool (31). Less commonly, histopathologic examination of intestinal and gallbladder specimens may be useful for demonstrating the intracellular parasites and for ruling out noninfectious conditions. Cryptosporidium parasites are observed in locations directly below the cell membrane and appear to “sit” on the luminal surface of the epithelium, whereas C. cayetanensis and C. belli are seen within the cytoplasm of the cells (31).
Antigen detection

As mentioned previously, antigen detection methods offer significantly improved detection of Cryptosporidium in stool, with immunofluorescence microscopy being widely considered to be the test of choice for routine diagnostic testing, followed closely by enzyme immunoassay-based tests (31, 44). There are several commercially available kits available.

Molecular methods

Until recently, NAATs for the coccidia were used primarily in public health laboratories and in the research setting; however, several assays are now commercially available, including FDA-cleared multiplex assays for gastrointestinal pathogens (33).

Treatment

Treatment of coccidial infections is generally straightforward in immunocompetent patients since disease is usually self-limited and responds to supportive or antiparasitic therapy; however, treatment can be challenging in immunocompromised hosts. Cryptosporidiosis is perhaps the most difficult of the coccidia to treat in immunocompromised hosts given that the only approach with proven efficacy is immune restoration. These individuals do not respond to the standard 3-day course of nitazoxanide that is recommended for immunocompetent hosts (34), and a systematic review and meta-analysis of literature on treatment of cryptosporidiosis in immunocompromised individuals also showed no benefit from treatment with paromomycin (57). In one case, Palmieri et al. demonstrated a favorable response to paromomycin and azithromycin in a patient with AIDS and pulmonary cryptosporidiosis (58), suggesting that there may be a role for combination therapy.

In contrast to cryptosporidiosis, immunocompromised hosts with cyclosporiasis and cystoisosporiasis generally respond to conventional therapy with trimethoprim-sulfamethoxazole (TMP-SMX), but prolonged treatment courses or use of secondary prophylaxis for immunocompromised patients may be indicated (34). A study by Verder et al. demonstrated that 100% of HIV-infected patients with cyclosporiasis experienced resolution of symptoms when treated with TMP-SMX, compared to only 87% (10 of 11) of HIV-infected patients treated with ciprofloxacin (59). Furthermore, all patients treated with TMP-SMX in this study stopped shedding detectable C. cayetanensis oocysts in their stool, whereas oocysts were still detectable in four of the 11 patients in the ciprofloxacin-treated group, thus supporting TMP-SMX as the preferred treatment. In patients with recurrent or persistent infection, it may be necessary to continue therapy indefinitely (31).

Control

Infections with coccidia and Cryptosporidium are prevented and controlled primarily through institution of improved sanitation and hygiene. Immunocompromised patients should be counseled and should also avoid contact with farm animals, contact with human or animal

**FIGURE 3** Round oocysts of Cryptosporidium sp. stained with a modified acid-fast stain. Note that not all oocysts stain equally and that sporozoites can occasionally be seen within some oocysts (1000x).

**FIGURE 4** Round oocysts of Cyclospora cayetanensis stained using a modified acid-fast stain (1000x). They have a similar appearance to the oocysts of Cryptosporidium but are larger (8 to 10 μm in diameter). Note that not all oocysts stain uniformly, producing unstained “ghost” cells.
feces, and inadvertent ingestion of water when swimming in fresh or man-made water sources, since these are potential sources of Cryptosporidium infection (44, 48, 60). Oocysts of all three parasites are resistant to routine chlorination and are unlikely to be inactivated by iodine water purification tablets or water halogenation methods and hand sanitizers. When visiting endemic areas, drinking water should be boiled and fruits and vegetables should be peeled prior to consumption (31). There are no available vaccines at this time.

Microsporidia (Microsporidiosis)

Introduction

The term “microsporidia” refers to multiple genera of obligate intracellular spore-forming organisms in the phylum Microsporidia (61). These organisms have gone through several taxonomic reclassifications since their initial discovery in 1857, and though previously thought to be protozoa, they are now considered to be most closely related to fungi (62). Testing for these organisms is still commonly performed in the parasitology laboratory and they are therefore included in this chapter.

Microsporidia are capable of infecting a large number of vertebrate and invertebrate hosts and can involve virtually any organ. Only a handful of human cases had been reported prior to 1985, but this changed significantly with the onset of the HIV epidemic and the recognition of gastrointestinal and disseminated microsporidiosis in patients with AIDS. We now know that microsporidia are important opportunistic pathogens in SOT recipients, while causing self-limited diarrhea in travelers, children, the elderly, and contact lens wearers, who can experience corneal infection (6, 61). The two most common species to cause human intestinal disease are Enterocytozoon bieneusi and Encephalitozoon intestinalis, while other Encephalitozoon spp. and members of several different genera (e.g., Anneliacia, Pleistophora, Trachipleistophora, and Vittaforma) infect organs throughout the body (61, 63).

Life cycle and transmission to humans

Transmission is thought to be through the fecal–oral route and possibly through sexual contact and exposure to infected animals (64). Environmentally resistant spores likely enter the body through ingestion or inhalation and then infect epithelial cells in the small intestine or lungs, respectively (65). Spores are capable of injecting their infectious sporoplasm into a host cell through use of an extruded polar tube. New spores then form within the host cell until the cytoplasm swells and the cell bursts. Newly released spores may infect neighboring cells or disseminate to other organs (65).

Pathophysiology

Similar to infection with Cryptosporidium and the coccidia, intestinal microsporidial infection leads to enterocyte damage, reduced mucosal surface area and malabsorption. The jejunum is the most common site of infection in the small intestine, while the large intestine is relatively spared (66). Intestinal and other organ involvement may lack an accompanying immune response, particularly in immunocompromised patients, or may be associated with granulomatous inflammation.

Epidemiology

Heightened awareness and development of improved diagnostic techniques have led to the increased recognition of microsporidiosis in both immunocompetent and immunocompromised hosts, although the rates of detection vary widely by the population tested and the methodologies used. Seroprevalence rates among pregnant women, blood donors, slaughterhouse workers and patients with unknown causes of diarrhea have ranged from 1.3 to 22%, whereas higher rates (up to 42%) have been detected in MSM (61). Use of molecular-based techniques have allowed for higher rates of detection in stool, urine and respiratory specimens from both asymptomatic and symptomatic patients, regardless of immune status, thus complicating our understanding of microsporidia epidemiology (33, 67–70).
It is now well-accepted that microsporidiosis is a common cause of diarrhea in immunocompromised patients, particularly in patients with AIDS and a CD4 count of ≤100 cells/μl (6, 61, 71). Patients with HIV are also at increased risk for ocular microsporidiosis. Prior to the use of combination antiretroviral therapy, prevalence rates of intestinal microsporidiosis in patients with HIV infection ranged from 2 to 50% (64). These rates have since declined with improvements in HIV therapies, but microsporidiosis remains an important cause of morbidity in patients with limited access to combination antiretroviral therapy. Other immunocompromised individuals at risk for symptomatic microsporidiosis include SOT and stem cell transplant (SCT) recipients, the elderly, patients with diabetes, and those undergoing chemotherapy for malignancy (64).

Clinical presentation
The clinical presentation of microsporidiosis varies with the infecting species and organ system involved. Immunocompetent individuals such as travelers and children generally present with self-limited watery diarrhea that responds to albendazole therapy. In contrast, immunocompromised patients may suffer from chronic watery diarrhea, weight loss, malaise and fever (61). Patients with AIDS may have four to eight bowel movements each day with associated nausea, anorexia, dehydration and mild hypokalemia (31). *Enterocytozoon bieneusi* is the most common cause of intestinal microsporidiosis in patients with AIDS and may rarely also involve the pulmonary and biliary systems. The *Encephalitozoon* spp. also commonly cause persistent diarrhea in this population but are more likely to disseminate to the lung, brain, liver, muscle, eye, and respiratory tract (61). In particular, microsporidia may involve the transplanted organ and contribute to graft failure (63). Manifestations of disseminated disease may include hepatitis, myositis, interstitial nephritis, sinusitis, tracheobronchitis and encephalitis. Ocular microsporidiosis may manifest as keratoconjunctivitis and stromal keratitis (61).

Diagnosis
Microsporidia are most commonly detected in the clinical laboratory by light or fluorescent microscopic examination of clinical specimens such as stool, urine, and respiratory secretions. These methods allow for identification of microsporidial spores but will not provide species-level differentiation, which instead requires examination of the parasite ultrastructure (i.e. transmission electron microscopy) or molecular amplification techniques (33). Immunofluorescence assays are also commercially available for some species. Serology tests are not useful for routine clinical diagnosis since they are not capable of differentiating between active infection, asymptomatic colonization, and past exposure (31).

Direct detection by microscopy
Light microscopic examination of stained preparations is a commonly used method for diagnosing microsporidial infections (65). The most commonly used stain is the chromotrope 2R method (e.g., Ryan’s trichrome-blue modification of the Weber trichrome method), which allows for identification of microsporidia spores from background fecal elements (72). The spores stain a deep red and often have a central band-like stripe (Fig. 6). A rapid “quick-hot Gram chromotrope” has also been described (73). Nonspecific optical brighteners such as calciofluor white, Uvitex 2B, and Fungi-Glo bind the chitin in microsporidial spores and are useful for rapid screening; however, they will also stain yeasts, which can lead to false-positive results if shape is not noted and size is not monitored by ocular micrometer. A number of histochemical stains are also used for identification of microsporidial spores in formalin-fixed paraffin embedded tissue sections, including Gram, acid-fast and Warthin-Starry stains; these are illustrated in a recent review (63). Spores are elliptical and vary in size, with the spores of *E. bieneusi* being among the smallest.

**FIGURE 6** Microsporidial spores of *Enterocytozoon bieneusi* in a concentrated fecal specimen demonstrating a characteristic band-like stripe (trichrome blue stain, 1000X). These tiny spores measure 0.8 to 1.4 μm in length and are therefore challenging to identify on standard microscopic preparations.
(<1.5 μm) and *Encephalitozoon* spores being only slightly larger (up to 2.5 μm). In contrast, some of the extraintestinal species (e.g., *Annacalia*) are significantly larger and may be mistaken for small yeasts such as *Histoplasma capsulatum* and *Candida glabrata* (63).

Transmission electron microscopy is considered the reference standard for species-level identification and taxonomic classification, but it is rarely performed in the clinical laboratory. Features such as the spore size, number of coils of the polar tubule and host–parasite relationship within the cell are used to identify and differentiate microsporidial species. Considerable expertise is required for accurate interpretation.

**Antigen detection**  
A newer approach for microscopic detection of microsporidial spores uses monoclonal or polyclonal antisera for detection of specific species by IFA. Unfortunately, reagent availability is limited (31).

**Molecular methods**  
A number of conventional and real-time PCR assays have been described for specific species or groups of microsporidia. Although they generally provide superior sensitivity to conventional microscopy-based techniques, they are mostly limited to the research or reference laboratory setting. There are no FDA-cleared assays for microsporidia at this time (33, 74).

**Culture**  
Microsporidia can be cultivated in a number of cell lines, including some that are used for conventional viral culture. However, this technique is rarely performed for clinical diagnostic purposes and can take 3 to 6 weeks for growth to be detected (75).

**Treatment**  
Oral albendazole is the mainstay for treatment of intestinal microsporidiosis due to *E. intestinalis* and disseminated microsporidiosis, while oral fumagillin is recommended for treating *E. bieneusi* intestinal infection (34). Unfortunately, oral fumagillin is not available in the United States and may cause significant bone marrow toxicity. Both fumagillin eye drops and oral albendazole are recommended for treating ocular microsporidiosis (34).

**Control**  
Although the sources of human infection are not completely understood, contact with water has been repeatedly identified as a risk factor in epidemiologic studies (6). Other potential sources of infection include animal contact and human-to-human transmission. Also, immunosuppressed individuals should be counseled regarding infection and should also avoid contact with potentially infected animals, contact with human or animal feces, and inadvertent ingestion of water when swimming in fresh water sources (31).

**BLOOD AND TISSUE PROTOZOA**  
The blood and tissue parasites that have the largest impact on immunocompromised hosts include the free-living amebae, *Toxoplasma gondii*, *Leishmania* species, *Trypanosoma cruzi*, *Plasmodium* species, and *Babesia* species, each of which will be discussed below. Of the protozoa found in the blood, those causing Chagas disease, malaria and babesiosis have the greatest potential impact on immunocompromised patients.

**Free-Living Amebae**  
*(Granulomatous Amebic Encephalitis)*  
**Introduction**  
The free-living amebae (FLA) are found in both the natural environment and in man-made facilities and may occasionally be human parasites (76). While many FLA have been identified, the three main genera associated with human disease are *Acanthamoeba* spp., *Balamuthia mandrillaris* and *Naegleria fowleri* (77). The following sections will focus on *Acanthamoeba* spp. and *B. mandrillaris* as infection with *N. fowleri* is exceedingly rare and is not common in immunocompromised hosts. Both *Acanthamoeba* species and *B. mandrillaris* cause granulomatous amebic encephalitis (GAE), primarily in immunocompromised hosts, while *Acanthamoeba* spp. can also cause a localized infection of the eye, referred to as amebic keratitis (AK). Both pathogenic and innocuous FLA can also harbor a variety of clinically significant pathogens, including *Legionella pneumophila*, *Listeria monocytogenes*, *Vibrio cholerae*, and enterovirus and are thus important reservoirs for nosocomial infections in hospitals with contaminated water sources (78–80).

*Acanthamoeba* and *Balamuthia* species are in the Orders Centramoebida and Acanthamoebidae, respectively (81). Sequence analysis of the small-subunit rRNA has identified 17 different *Acanthamoeba* genotypes (T1 to T17), of which the T4 genotype, encompassing *A. castellanii*, *A. lugdunensis*, *A. mauritaniensis*, *A. polyphaga*, *A. rhysodes*, *A. royi* and *A. triangularis*, predominates among clinical isolates (82). *B. mandrillaris* (previously known as *Leptomyxid amoeba*) was originally considered an innocuous environmental FLA but...
was later recognized as a cause of fatal meningoencephalitis in a mandrill baboon from the San Diego Zoo in 1990 (83). Additionally, B. mandrillaris was described as causing serious infections in solid organ recipients following transplantations of infected tissue (84). Currently, B. mandrillaris is the only known species within the Balamuthia genus.

Epidemiology
Both FLA have a worldwide distribution. Acanthamoeba spp. are the most widespread and prevalent FLA and have been isolated from many natural environments (e.g., soil and vegetation, fresh, brackish, and sea water), dental offices, dialysis units, contact lenses, air conditioning units, mammalian cell culture lines, and from healthy humans (81). As a result of their widespread distribution, human exposure to Acanthamoeba spp. is common, and seroprevalence studies have shown that approximately 80% of otherwise healthy individuals are seropositive for antibodies to this amoeba (85). In comparison, B. mandrillaris is the most common amoeba isolated from soil and dust and is less commonly recovered from water sources, thus suggesting that the risk for exposure to B. mandrillaris may be more associated with activities such as agricultural work, gardening, mountain biking, and other soil-disruptive activities (86, 87). The seroprevalence is also high for this organism, indicating that human exposure is common. The majority of B. mandrillaris cases in the United States have occurred in the Southwest (i.e., Arizona, California and Texas), while in Latin America, Peru has reported the highest number of cases, with 53 occurring between 1975 and 2011 (88). In 1998, the California Encephalitis Project was established to test unexplained cases of encephalitis for both common and uncommon infectious agents, including for Balamuthia. Through the California Encephalitis Project, between 1997 and 2007, 10 cases of GAE were identified in the United States, and all but one of these patients expired (89).

GAE is most common in patients with immunocompromising conditions such as HIV, malignancy, corticosteroid treatment, systemic lupus erythematosus, pregnancy, hematologic disorders, tuberculosis, diabetes, and alcoholism (81, 90, 91). This infection has become more widely recognized in AIDS patients, particularly those with low CD4+ T-cell counts (<200 cells/μl). Unfortunately, diagnosis is often made postmortem due to fulminant disease progression and mortality within approximately one month of symptom onset (92). Interestingly, GAE due to B. mandrillaris has also been documented in otherwise healthy hosts, including young children and the elderly (93), with approximately half of the cases reported from Peru and occurring in children (94).

Life cycle and transmission to humans
The life cycle of both FLAs involves two forms: an actively dividing trophozoite stage and a dormant cyst stage. Trophozoites are uninucleate, divide through binary fission, and feed on surrounding bacteria and algae through phagocytosis or pseudopod formation. If environmental conditions become challenging (e.g., desiccation, osmolarity imbalance, nutrient deprivation, etc.), trophozoites will encyst and become resistant to most antimicrobials and biocides. Cysts remain viable for years and will yield pathogenic trophozoites. Of note, the virulence of excysted trophozoites decreases with duration of encystment (95). While the trophozoite stage is the infectious form, infection can also be acquired through contact with cysts which will subsequently excyst under amenable conditions (96, 97). Exposure to both organisms is thought to be due to inhalation of the organism into the lower respiratory tract and/or contamination of broken skin. Acanthamoeba spp. can also infect the eye and cause localized disease (e.g., keratitis). CNS invasion and GAE typically occur following hematogenous dissemination of the amoebae from either a cutaneous lesion or from the respiratory tract.

Pathophysiology
Pathogenicity centers around the organism’s ability to adhere to mucosal surfaces and invade tissues. Acanthamoeba spp. produce a variety of proteases, including alkaline cysteine proteinases and serine proteases, and release oxygen radicals which may facilitate cytolysis of tissues, including the corneal epithelium, invasion of the extracellular matrix, and dissolution of the corneal stromal matrix (81, 98). In comparison, the pathophysiology of B. mandrillaris is not well understood. Both in vitro and in vivo studies suggest that the organism produces a significant quantity of proteolytic enzymes to degrade the extracellular matrix around cells, allowing for rapid tissue invasion, and also appears to stimulate a strong inflammatory response (e.g., IL-6, TNF-α, IL-1, IL-8, etc.) leading to blood-brain barrier permeability (96, 99). Further studies are needed to fully elucidate the pathogenesis of this organism.

Clinical presentation
The incubation period for Acanthamoeba spp. and B. mandrillaris remains unknown, although from the available cases, the range can vary from days to several weeks (100).
Amebic keratitis
Among otherwise healthy individuals, keratitis, uveitis and corneal ulcerations associated with *Acanthamoeba* infections are most commonly observed, particularly among contact-lens (hard and soft lens) wearers. AK is often misdiagnosed as herpes simplex or bacterial or fungal keratitis and may be present as a secondary or opportunistic infection in patients or could be missed entirely (101, 102). Severe pain, nonhealing corneal ulcers, and the presence of ring infiltrates in the eye are clinical signs that should alert an ophthalmologist to the possibility of amebic infection. Outbreaks of AK have been linked to contaminated multipurpose contact lens solution; however, homemade contact solutions and generally unhygienic maintenance of contact lenses are also significant risk factors for infection (103). Immunosuppressed individuals can develop AK, and therefore *Acanthamoeba* infection should be included in the differential diagnosis of uveitis in AIDS patients in particular (104).

Cutaneous disease
Cutaneous *Acanthamoeba* infection is a rare, opportunistic infection, often resulting from hematogenous dissemination or traumatic implantation and is most commonly observed in AIDS patients (93, 105, 106). Lesion appearance can vary from hard, erythematous, subcutaneous nodules to significant ulcerations and can occur throughout the trunk, extremities, and on the face. Similarly, cutaneous *B. mandrillaris* infections present as single to multiple plaques or nodules with a rubbery to hard consistency. If left untreated, trophozoites and/or cysts from cutaneous FLA lesions can disseminate to the CNS, leading to GAE.

**GAE**
GAE is an insidious and chronic disease of the CNS, which develops over weeks to months following hematogenous dissemination of *Acanthamoeba* spp. or *B. mandrillaris* from a primary infection site such as the skin or lungs. At the onset, patients with GAE typically experience lethargy, acute onset of fever, headache, and neck pain. Subsequent symptoms include confusion, dizziness, drowsiness, nausea, vomiting, headache, lethargy, stiff neck, seizures, and at times hemiparesis (81, 97). The cerebral hemispheres are most often involved, showing edema and hemorrhagic necrosis within the temporal, parietal, and occipital lobes. Neuroimaging findings indicate heterogeneous, hyperdense, nonenhancing, space-occupying lesions, typically involving the cerebral cortex and subcortical white matter. However, these findings are nonspecific and can suggest CNS neoplasm, tuberculoma or septic infarcts (94). Infection may be associated with a granulomatous response, although this is generally minimal or even absent in AIDS patients. A chronic inflammatory exudate, composed mainly of polymorphonuclear leukocytes and mononuclear cells, may also be observed over the cortex. Cerebrospinal fluid (CSF) examination of patients with GAE reveals elevated protein, depleted glucose levels and abundant lymphocytes and polymorphonuclear leukocytes. Pleocytosis may be absent in AIDS patients (81).

The duration of the clinical course can vary significantly, from a few days to several months. Mortality rates are high, generally exceeding >95%, regardless of immune status (93, 94).

**Diagnosis**
Diagnosis of GAE remains a challenge and is often only identified during postmortem examination. Identification of FLA trophozoites and/or cysts through direct microscopy on raw specimens, histopathology and/or culture are the primary diagnostic methods, regardless of disease manifestation. Serologic evaluation is not routinely available and not generally recommended due to the high seroprevalence of antibodies to the FLA. Detection of FLA DNA by real-time PCR may provide increased sensitivity and specificity over traditional identification methods but is only available from the CDC and select national reference laboratories (107, 108). The preferred specimens for diagnosis of AK due to *Acanthamoeba* spp. are either corneal scrapings or biopsies; corneal or conjunctival swabs should be avoided. While *Acanthamoeba* spp. may be cultured from the implicated contact lens or contact case, a positive result is only suggestive of infection, as *Acanthamoeba* spp. may be recovered from up to 8% of contact lenses collected from otherwise asymptomatic individuals (93, 109, 110). Biopsy specimens should be submitted to the microbiology and surgical pathology laboratories for direct microscopy and histopathology, while CSF should also be submitted for culture.

**Direct detection by microscopy**
Direct microscopy of CSF or tissue wet preparations should be performed immediately to look for mobile trophozoites and cysts. Trophozoites of *Acanthamoeba* range in diameter from 25 to 40 μm and are distinguished from other amoeba by the presence of acanthopodia (i.e., spiny surface projections; seen best in wet preparations). They also have a distinct contractile vacuole, which regulates the cytoplasmic fluid level,
and a large karyosome within a single cellular nucleus. *B. mandrillaris* trophozoites are slightly larger (30 to 60 μm) and have extensive branching pseudopodia. They also have a single nucleus with a central karyosome, though binucleate and multinucleated forms have been observed. *Acanthamoeba* spp. cysts are generally round, range in diameter from 13 to 20 μm and have a wrinkled, double cell wall. The cysts of *B. mandrillaris* may be slightly larger (10 to 30 μm in diameter). Both have a single nucleus and large karyosome (94).

Examination of formalin-fixed, paraffin-embedded tissue is also useful for establishing a diagnosis in cases of AK and GAE (Figs. 7 and 8). In cases of GAE, cysts and trophozoites are generally found in necrotic regions of the brain and are often closely associated with blood vessels, reflecting the hematogenous nature of the infection. The two FLA are indistinguishable in this setting and therefore require use of molecular techniques for definitive identification.

Despite the characteristic morphology of the FLA trophozoites, they may be misidentified due to their resemblance to macrophages and other host cells. To improve the sensitivity of the CSF examination, CSF sediment should be concentrated using centrifugation (250 × g for 10 min), placed on a slide and fixed in methanol. Smears can then be stained with Giemsa-Wright or trichrome stains to better discriminate between amoebae and host immune cells. The primary feature used for differentiating *Acanthamoeba* spp. from macrophages is the nucleus; *Acanthamoeba* spp. have a small, generally round nucleus with a large central karyosome, while macrophages have a larger, irregular-shaped nucleus without a large karyosome or nucleolus. Periodic acid-Schiff, Gomori-methenamine silver and calcofluor white stains can also be used to identify *Acanthamoeba* cysts (35, 81).

**Culture**

Culture for *Acanthamoeba* spp. can be performed on freshly collected CSF, brain tissue or other biopsy material. Traditionally, the specimen is plated on non-nutrient agar (1.5% agar) plates that are overlaid with

---

**FIGURE 7** *Acanthamoeba* spp. trophozoites and cysts in a skin biopsy of an ulcerating skin lesion. Organisms are seen invading blood vessels in the dermis (left, H&E, 200×; arrow shows lumen of blood vessel). Higher magnification shows trophozoites with a small nucleus and large karyosome (top right) and cysts with a characteristic hexagonal double cell wall (bottom right) (H&E, 1000×).
either *Escherichia coli* or *Enterobacter aerogenes* as a nutrient source for the actively replicating trophozoites. The specimen is incubated at room temperature and screened daily for up to one week. In comparison, *B. mandrillaris* will not grow on nonnutrient agar overlaid with *E. coli* or *E. aerogenes* and instead requires eukaryotic cell culture (99). The FLA can be cultured on a variety of mammalian cell lines, including HEp-2 and VERO cells, and can produce cytopathic effects similar to those caused by viruses, thus requiring experienced personnel for accurate identification (77).

**Treatment**

In cases of amoebic keratitis, early detection prior to invasion of the organism into deeper corneal tissue is critical to minimize vision loss. Superficial infections have successfully been treated with debridement and/or topical application of propamidine isethionate and dibromopropamidine or chlorhexidine and propamidine (111, 112). Intravenous imidazoles (e.g., itraconazole, ketoconazole, and fluconazole) have also been applied, though with limited success. Invasive cases of keratitis typically require prolonged treatment, in some cases for over a year, and patients require monitoring for recurrence as treatment failure is not uncommon. *In vitro* susceptibility testing may also be helpful in such cases (113).

There is currently no standard therapy against disseminated *Acanthamoeba* infections or GAE, and many different combination regimens have been attempted on a case-by-case basis. While some of these treatments have been effective, the majority of patients with severe disease succumb to infection (97). Combination therapy has included sterol-targeting azoles (clotrimazole, miconazole, ketoconazole, fluconazole, and itraconazole), pentamidine isethionate, flucytosine, and sulfadiazine and is nicely reviewed by Marciano-Cabral and colleagues (81). While corticosteroids have been used to counteract cerebral edema and inflammation, steroids should be avoided as they tend to exacerbate the *Acanthamoeba* infection (81).

Treatment for *B. mandrillaris* infections is not standardized, and similar to infections with *Acanthamoeba*, regimens are based on *in vitro* susceptibility data and case reports. *B. mandrillaris* shows *in vitro* susceptibility to pentamidine isothiocyanate. Review of the case literature indicates that patients have survived on various multidrug therapeutic regimens including 1) flucytosine, pentamidine isethionate, fluconazole, sulfadiazine, and a macrolide; or 2) miltefosine, albendazole, and fluconazole (114, 115). Notably, the latter regimen has been utilized repeatedly, with some success in cases of *B. mandrillaris* infection in Peru.

**Control**

As the FLA are virtually ubiquitous in both naturally occurring and manmade environments, exposure to these agents is inevitable and control options are limited. Therefore, education of immunocompromised patients regarding high-risk activities which may lead to *Acanthamoeba* and *B. mandrillaris* infection (e.g., improper cleaning of contact lenses, excessive exposure to disrupted soil, etc.) is strongly encouraged.

**Toxoplasma gondii** (Toxoplasmosis)

**Introduction**

*Toxoplasma gondii* is an obligate, intracellular protozoan parasite in the phylum Apicomplexa that is infectious to a wide variety of animals. Individuals particularly at risk for severe infection and sequelae include individuals with a latent infection who undergo immunosuppression, SOT recipients and pregnant women who develop a primary infection during pregnancy. Serology remains the mainstay for diagnostic testing; however it is not without limitations, including the inability to differentiate past from present infection and insensitivity in immunocompromised patients.

**Epidemiology**

Although seroprevalence rates vary significantly from country to country and between socioeconomic groups, approximately 25 to 30% of the world’s population is
thought to be infected with *T. gondii* (116). While the seroprevalence rate for antibodies to *T. gondii* ranges from 10% to 22% in the United States, seropositivity rates as high as 75% have been documented in El Salvador and France (117). Additionally, individuals in low socioeconomic circumstances, regardless of country of residence, typically show higher seropositivity rates than individuals in higher socioeconomic groups. Notably, over the last decade, seroprevalence rates in many countries have been declining, likely due to improved sanitation practices and better understanding of the risk factors associated with *T. gondii* infection (116).

**Life cycle and transmission to humans**

The life cycle of *T. gondii* involves three different forms: rapidly dividing, invasive tachyzoites; slowly dividing cyst bradyzoites; and an environmentally hardy oocyst containing infectious sporozoites. Classically, the *T. gondii* life cycle alternates between a definitive felid (cat) host and an intermediate host. However, unlike other apicomplexan members, *T. gondii* can also cycle between intermediate hosts via carnivorism or vertical transmission. Cats become infected when they ingest tissue contaminated with *T. gondii* cysts. These cysts infect enterocytes and undergo sexual reproduction to form the oocysts. Oocysts are excreted in the feces into the environment and internally sporulate, becoming infective within 2 to 3 days of being released. Infection of the intermediate host (e.g., rodents, herbivores, humans) typically occurs through ingestion of oocysts in contaminated food or water, although humans and other animals can also become infected by eating raw or undercooked meat containing *T. gondii* cysts. Following ingestion, parasites penetrate the intestinal lining and differentiate into tachyzoites, which can invade virtually any host cell, replicate, and disseminate throughout the body. The parasite will convert to the bradyzoite form within 7 to 10 days of infection in an immunocompetent host, forming large cysts in muscle tissue or the brain. These cysts can remain in a dormant state for the life of thehost or until immunosuppression is induced. In contrast, tachyzoites may replicate unchecked within host cells in immunocompromised hosts, resulting in cell rupture and infection of adjacent cells. Other less common means of *T. gondii* transmission are via contaminated blood products or organs and by transplacental transmission (97).

**Pathophysiology**

The host immune response to *T. gondii* infection has been studied extensively in both mice models and humans over the last two decades. *T. gondii* induces a strong Th-1 response leading to production of proinflammatory cytokines, including IL-12, IFN-γ and TNF-α, which ultimately help to control the parasite burden (118). Therefore, individuals with impaired cellular immunity, due to HIV/AIDS or management with immunosuppressive therapies, are at highest risk for severe manifestations of toxoplasmosis. These patients are more likely to develop disease due to reactivation of a dormant infection (i.e., rupture of a cyst) versus acquisition of a new infection. For patients with HIV infection, the risk of toxoplasmosis increases significantly when CD4+ T cell counts fall below 100 cells/μl. Prior to use of antiretroviral therapy, the most common manifestation of disseminated toxoplasmosis in this patient group was toxoplasmic encephalitis. Since effective antiretroviral therapies have become widely available, the incidence of toxoplasmic encephalitis (TE) has dropped from four cases to one case per 100 person-years, though dissemination to other organs, including primarily the lungs, eyes and heart, continues to occur. For organ recipients, both reactivation of a latent infection or transplantation of an infected organ into a seronegative recipient can lead to severe disease, with hematopoietic SCT patients at highest risk (116).

**Clinical presentation**

For approximately 90% of otherwise healthy individuals, infection with *T. gondii* is entirely asymptomatic. The remaining 10% of patients typically experience self-limiting, nonspecific symptoms, including fever and lymphadenitis, which may last for several weeks and mimic other infectious diseases (117). The infection subsequently becomes latent, and patients remain asymptomatic unless they become immunosuppressed. Chorioretinitis can also occur in immunocompetent patients during the second or third decade of life, when cysts may rupture and lesions develop in the eye (119).

In immunocompromised patients, toxoplasmosis can develop subacutely over weeks to months or can present acutely with rapid deterioration and death over days to weeks. Risk factors for development of toxoplasmosis include uncontrolled HIV/AIDS, organ transplant recipients, cancer patients, and use of immunosuppressive drugs. The most common manifestation of toxoplasmosis among immunocompromised individuals is TE, which can present with motor deficits, sensory abnormalities, seizures, lethargy, fever, and altered mental status (120, 121). Chorea has also been associated with TE and AIDS patients, and while not unusual in this patient population, the causes are not entirely clear.
(122). It is therefore recommended that careful neuro-radiological and clinical evaluation be undertaken for AIDS patients presenting with chorea to rule out *T. gondii* infection. Secondly, toxoplasmosis should be considered in young patients presenting with chorea without a family history of movement disorders. Overall, these general symptoms, along with CT and MRI imaging studies, which may reveal ring-enhancing lesions, are not specific for TE, requiring providers to consider other causes of disease, including Cryptococcus spp., Aspergillus spp., Nocardia spp., and progressive multifocal leukoencephalopathy, among others (117).

Aside from TE, immunocompromised patients who develop toxoplasmosis may present with chorioretinitis, pneumonitis, myocarditis, cystitis, hepatitis or respiratory failure and multiorgan distress. Pneumonia due to *T. gondii* is rare, though it is reported more commonly in SCT recipients and AIDS patients (117). Gastric toxoplasmosis, also uncommon, can present as a primary manifestation of *T. gondii* infection in AIDS patients (123, 124).

In transplant recipients, disease severity depends on donor and recipient *T. gondii* exposure status, the type of organ transplanted, and the extent of recipient immunosuppression. Disease can occur from reactivation of a latent infection or from acute, primary infection that is acquired directly from the transplanted organ. SCT recipients who are seropositive for antibodies to *T. gondii* prior to transplantation are at highest risk for disseminated toxoplasmosis, primarily due to reactivation of a latent infection (125–129). All potential transplant recipients should therefore be tested for Toxoplasma-specific IgG antibodies to determine their antibody status.

Finally, while prior maternal infection with *T. gondii* poses little threat to the fetus, primary infection with *T. gondii* immediately before conception (i.e., within 3 months) or during gestation carries significant risk to the fetus because circulating parasites can cross the placenta. Vertical transmission has been documented among chronically infected females who become significantly immunosuppressed (e.g., acquire HIV, initiate corticosteroid treatment, etc.) during pregnancy (130). The severity and frequency of congenital transmission vary greatly relative to gestational age at the time of maternal infection and are inversely related. Briefly, while the incidence of *T. gondii* transmission is low during the first (9%) or second (27%) trimester, congenital infection often results in severe fetal toxoplasmosis for approximately 78% and 20% of cases during the first and second trimester, respectively (117). Ultrasonographic findings in cases of congenital *T. gondii* infection may include intracranial calcifications, hydrocephalus and microcephaly, and fetal death, which occurs in 2% to 5% of cases. Conversely, the frequency of vertical transmission during the third trimester is highest (59%), though the majority of newborns (90%) do not exhibit any clinical symptoms. If not treated, babies may develop growth delays and chorioretinitis later in life (117). Importantly, if *T. gondii* infection is recognized early, around the time of conception or within the first 2 weeks of gestation, and the mother is initiated on spiramycin therapy, vertical transmission is inhibited.

### Diagnosis

Multiple methodologies are available for the diagnosis of toxoplasmosis, including serology, molecular methods to detect *T. gondii* nucleic acid, and microscopy. Choosing the most appropriate testing method depends largely on the patient’s immune status. Serologic testing is generally used for otherwise healthy individuals, as these patients will have detectable antibody levels and results will allow for differentiation from infection with other infectious agents (e.g., cytomegalovirus, Epstein-Barr virus, HIV) or noninfectious conditions. In contrast, serologic testing is generally avoided for evaluation of immunosuppressed patients as their antibody responses may be unreliable and lead to false-negative results; detection and monitoring of *T. gondii* infection in this patient population is primarily achieved by molecular methods and/or microscopy.

#### Direct detection by microscopy

Demonstration of free or intracellular tachyzoites or bradyzoite cysts in fluid or tissue is definitive evidence for an acute toxoplasmosis. Tachyzoites and bradyzoite cysts are the two parasite forms present during human infection and are detectable by the laboratory. Tachyzoites measure 4 to 5 μm in length by 1 to 2 μm in width and are classically crescent-shaped (Fig. 9). *T. gondii* tissue cysts range in diameter from 10 to 100 μm and are filled with hundreds of bradyzoites, measuring 3 to 4 μm in length by 1 to 2 μm in width.

As *T. gondii* can disseminate to almost any organ system, the most appropriate sample type to submit depends on clinical presentation. *T. gondii* parasites have been detected by direct microscopy or in histopathology sections from bronchoalveolar lavage fluid, blood, bone marrow, CSF and various tissues. Wright’s or Giemsa stains are routinely used to detect *T. gondii*, though specific anti-*T. gondii* immunoperoxidase or...
fluorescent antibody stains may be available through select reference laboratories and the CDC (117, 131).

While microscopy methods are fairly rapid, inexpensive and allow for definitive identification, the sensitivity of these techniques is dependent on the quality of the specimen submitted (i.e., proper site biopsied) and level of parasitemia at the time of collection. Therefore, a single negative result in a high-risk patient exhibiting symptoms should not be used to exclude toxoplasmosis. Finally, while detection of *T. gondii* cysts in biopsy material is indicative of latent infection, this finding may be misleading as the organism may be identified but may not be the etiologic agent of acute disease. This caveat should be considered if the patient does not improve on anti-Toxoplasma treatment regimens.

**Molecular methods**

NAATs targeting numerous repetitive genes of *T. gondii*, including the RE, B1 and AF146427 genes, have been used to detect *T. gondii* in various fluids (e.g., CSF, amniotic fluid, ocular fluid, etc.), as well as in fresh and paraffin-embedded tissue sections (131, 33). There are currently no standardized, FDA-cleared molecular assays for *T. gondii*, although a few CE-marked kits are available. The majority of NAATs rely on laboratory-developed methods, and the clinical sensitivity can vary from 64% to 100% (117, 127). This variability is due to differences in assay design, specimen collection, parasitemia at the time of collection, specimen storage, and transport prior to testing. Therefore, the absence of Toxoplasma DNA should not be used to exclude toxoplasmosis in high-risk patients. The sensitivity of NAATs may be increased by testing fresh or frozen biopsy material from these lesions, rather than CSF, as tachyzoites may not be present in the spinal fluid or may be present intermittently (132).

**Serology**

Detection of antibodies to *T. gondii* is primarily used to evaluate symptomatic immunocompetent individuals or pregnant women, and outpatients with uveitis or retinochoroiditis without a history of congenital infection. Serologic methods are also used to screen organ donors and recipients prior to transplant. Following primary infection of an otherwise healthy individual, anti-*T. gondii* IgM- and IgA-class antibodies are produced within the first week of infection, plateau approximately one month later, and decrease within the next 6 months. IgM antibodies become undetectable within 7 months for 25% of patients; however, they can remain detectable for a year or longer (133). IgG antibodies to *T. gondii* become detectable 2 to 3 weeks following the initial rise in IgM, plateau 2 to 3 months postinfection, and decrease over time but remain detectable for life (116). Diagnosis of congenital toxoplasmosis using serologic assays is challenging, because except for IgM-class antibodies, maternal antibodies readily cross the placenta. Therefore, it is recommended that paired maternal and neonatal serum be submitted for *T. gondii* antibody testing to a *T. gondii* reference laboratory. Testing for the presence of IgM-class antibodies using an immunosorbent agglutination assay and for IgA-class antibodies identifies up to 75% of congenitally infected babies (117). Additionally, molecular testing to detect *T. gondii* DNA in CSF, blood, or urine is useful for rapid identification of acute infection in newborns.

The highest risk for toxoplasmosis occurs in seronegative recipients of organs from seropositive (D+/R−) donors and trimethoprim/sulfamethoxazole prophylaxis has been shown to be highly effective in this setting (117). In general, serologic testing of immunosuppressed patients to detect active infection is of limited value as IgM levels may not be detectable in this patient population and the presence of IgG is indicative of chronic infection (116, 131). While demonstration of seroconversion or a 4-fold rise in antibody titers between acute and convalescent samples is suggestive of recent infection, this method is retrospective in nature, and for immunosuppressed individuals, rapid diagnosis and prompt treatment are necessary to minimize patient morbidity and mortality. For this patient population and particularly for hematopoietic SCT patients, the absence of specific antibodies does not exclude active disease.
Additionally, multiple case studies indicate that rising antibody titers in the absence of symptomatic infection may be misleading and a result of immune reconstitution, not active disease (117, 134). Should serologic evaluation be undertaken for immunosuppressed patients, direct detection methods should also be pursued.

**Treatment**

Treatment of toxoplasmosis involves combination therapy and, if possible, a reduction of immunosupression. The standard regimen for disseminated toxoplasmosis or TE is pyrimethamine and sulfadiazine, often administered with leucovorin (folic acid) to prevent hematologic side effects associated with pyrimethamine (97). Regimens are typically prescribed for up to 4 to 6 weeks following resolution of symptoms. Clindamycin has been used as an alternative to sulfadiazine for patients intolerant to sulfonamides (135). This regimen may be contraindicated in patients with a history of bone marrow suppression and severe allergic reactions to sulfonamides (136–138). The effectiveness of alternative drugs in immunocompromised patients, including azithromycin, clarithromycin and dapsone, has not been established, though should these drugs be employed, it is recommended that they be used in combination with pyrimethamine (117). Following treatment of acute infection, it is recommended that secondary prophylaxis using the same regimen be initiated and maintained for life or until immunosuppression is stopped. Discontinuation of *T. gondii* prophylaxis may be considered for AIDS patients in whom HIV viral loads have been well controlled for at least 6 months and who have a CD4* cell count of at least 200 cells/μl (139).

**Control**

As human infection can be acquired through ingestion or handling of infected meat or through ingestion of infective oocysts, hand washing following any potential exposure to oocysts is essential. Additionally, meat should be cooked until the internal temperature reaches 150°F (66°C). HIV-infected persons and other immunosuppressed individuals who are seronegative for *Toxoplasma* IgG should be counseled to protect themselves from primary infection by eating well-cooked meats and by washing their hands after high-risk activities, including soil and cat contact. Furthermore, cats kept as pets should be fed commercial or well-cooked food and should be kept indoors. If possible, immunosuppressed individuals should avoid changing litter boxes. Ideally, litter boxes should be changed daily and disinfected with boiling water (140). While research is ongoing to develop a vaccine to *T. gondii*, clinical trials have yet to begin.

**Leishmania Species (Leishmaniasis)**

**Introduction**

Members of the *Leishmania* genus are obligate, intracellular vector-borne protozoa which can cause a wide spectrum of disease, ranging from asymptomatic infection to diffuse or localized cutaneous leishmaniasis (CL), visceral leishmaniasis (VL) and mucocutaneous leishmaniasis (MCL). *Leishmania* spp. are members of the order Kinetoplastida due to the presence of a kinetoplast. The kinetoplast contains densely packed mitochondrial DNA within the mitochondrion. Other kinetoplastids of clinical importance are the *Trypanosoma* spp., discussed later in this chapter. Within the *Leishmania* genus are two subgenera, *Leishmania* and *Viannia*, each of which includes many species. Traditionally, *Leishmania* species have also been categorized according to geographic endemicity in the Old World (i.e., Africa, Asia, Europe) and the New World (i.e., Latin and Central America and the southern United States). The different *Leishmania* species, of which at least 21 are pathogenic to humans, have different tissue tropisms, are associated with different disease manifestations (i.e., CL, VL or MCL), and respond differently to treatment regimens. Therefore, complete identification of the infecting *Leishmania* species is clinically significant in some settings.

Briefly, CL is associated with five Old World species, including *L. infantum* (considered to be the same organism as the New World species *L. chagasi*), *L. tropica*, *L. major*, *L. aethiopica* and *L. donovani*, and over 11 different New World species, among which *L. braziliensis* is most common. MCL is almost exclusively caused by the New World species *L. braziliensis* and *L. panamensis*, while VL is primarily associated with *L. donovani* and *L. infantum*, though VL due to *L. tropica* has also been reported (141).

**Epidemiology**

Leishmaniasis remains a neglected tropical disease, with approximately 310 million individuals at risk for contracting the infection, 12 million people currently infected, and an annual incidence of 2 million new infections (141, 142). *Leishmania* infections, particularly VL, continue to increase in frequency among both HIV-positive and otherwise immunocompromised individuals, likely due to a general increase in the number of individuals maintained on immunosuppressive regimens, increased travel,
and dissemination of competent vectors into otherwise naive geographic regions (143–145). Leishmaniasis is considered endemic in 98 countries, and of the estimated 2 million new infections annually, 1.5 million are CL, 500,000 result in VL, and 50,000 individuals succumb to the infection. Despite the widespread distribution of the parasite, disease burden is largely concentrated to a few geographic foci (141).

Anthroponotic L. tropica, the major Old World species associated with CL, is distributed throughout India, Central and Western Asia, and into North Africa, whereas the multiple zoonotic Leishmania spp. associated with CL can be found from Argentina to the southern border of Texas. Importantly, 90% of all CL cases occur in Afghanistan, Iran, Saudi Arabia, Bolivia, Brazil, Colombia, Peru and Nicaragua. Regarding VL, anthropopotic L. donovani accounts for over 65% of all VL cases and is distributed in northeastern India, southeastern Nepal, central Bangladesh, and eastern Africa. L. infantum, the major zoonotic cause of VL, is primarily localized to the Mediterranean Basin, the Middle East, western Asia, and Brazil. Approximately 90% of all VL cases occur in Bangladesh, Brazil, Ethiopia, India, Nepal, and the Sudan. While these geographic foci are fairly well established, increasing reports of both CL and VL are emerging from Europe. This is likely a result of climate change, vector spread, increasing migration of infected individuals from endemic areas, and an increase in immunocompromised hosts (145).

Leishmania and HIV coinfections, particularly with L. infantum or L. donovani, have been on the rise throughout Europe and especially in countries surrounding the Mediterranean basin where these Leishmania spp. are endemic. Recently, the WHO and others indicated that approximately 90% of all Leishmania–HIV coinfections were reported from Spain, Italy, France, and Portugal (141, 146). Among these coinfected individuals, intravenous drug use rather than exposure to phlebotomine flies has been documented as the greatest risk factor for infection. European nations are also reporting an increasing number of leishmaniasis cases in organ transplant recipients. As the number of transplant procedures increases, the number of individuals on immunosuppressive regimens likewise increases, predisposing individuals for infection.

Life cycle and transmission to humans

Depending on the species, Leishmania can be considered either zoonotic, indicating that the natural reservoir is a nonhuman mammal (e.g., rodent, canid, etc.), or anthropoponic, indicating that humans are the reservoir host. While most species fall within one category or the other, depending on the geographic foci, some species (e.g., L. tropica) can be both zoonotic and anthropoponic. There are two major forms of the parasite, the extracellular promastigote found in the sand fly and the intracellular amastigote found in vertebrate hosts. While the classic mode of Leishmania transmission is through the bite of an infected sand fly, infection through contaminated needles shared among intravenous drug users, blood transfusion, organ transplantation and vertical transmission have also been documented, albeit rarely (97). Leishmania spp. are transmitted by two different genera of hemophagic phlebotomine sandflies: Phlebotomus species in the Old World and Lutzomyia species in the New World (141).

In most endemic foci, the vast majority of sand flies are uninfected; however, those that are infected are efficient vectors. Female sandflies acquire the parasite following ingestion of amastigote-infected macrophages or free, circulating amastigotes during a blood meal. Amastigotes differentiate into flagellated promastigotes in the sand fly, divide by binary fission and migrate to the fly proboscis. During the next blood meal, promastigotes are released into the host, and through the expression of specific surface molecules, such as gp63 and various lipophosphoglycans, bind to macrophage or other phagocytic cell receptors, stimulating phagocytosis (147, 148). Intracellular promastigotes subsequently differentiate into the nonflagellated amastigote form within infected reticuloendothelial cells where they multiply by binary fission, eventually destroying the cell and going on to infect new cells. While agents of CL are confined to and replicate almost exclusively within skin mononuclear phagocytes, species causing VL are engulfed by tissue macrophages and disseminate to organs of the reticuloendothelial system including the bone marrow, spleen and liver. Interestingly, agents of MCL initially cause cutaneous lesions but can subsequently disseminate through the lymphatics to the mucosal membranes of the nose, mouth, soft palate and throat (35, 97).

Pathophysiology

The host response to Leishmania infection is complex, with resistance or susceptibility to infection dependent on numerous factors, including parasite virulence, host genetic factors and immune status. Within minutes of release into the dermis, approximately 90% of the promastigotes are destroyed by the complement system, suggesting that infection is established by a small percentage of parasites (147). Since injected promastigotes...
are immediately phagocytosed by tissue-resident macrophages and dendritic cells, control of *Leishmania* spp. is largely dependent on activated macrophages and a robust Th1 response (145). Key proinflammatory cytokines associated with protection against leishmaniasis include IFN-γ, IL-12 and TNF-α. Use of TNF-α antagonists has garnered attention as an increasing number of VL cases, primarily from Europe, have been documented in these patients (145, 149). While an intact innate immune response is likely responsible for the asymptomatic infection of many individuals, overwhelming parasitemia or genetic factors weakening this response typically lead to severe manifestations of the disease (147, 150). Susceptibility to infection occurs more commonly in patients with a strong Th2 cell response, which is associated with macrophage quiescence, enabling *Leishmania* parasites to actively replicate within phagocytes (147). This is of particular concern among HIV/AIDS patients as 1) both HIV and *Leishmania* spp. infect the same cell population (i.e., macrophages and dendritic cells), significantly limiting the immune function of these cells; and 2) both agents avidly suppress the Th1 cellular immune response through mediation of cytokine production. Notably, HIV infection increases the risk of developing VL by 100 to 2,320 times in endemic areas, whereas VL promotes progression of HIV to AIDS and development of AIDS-defining illnesses (151, 152).

Finally, *Leishmania* organisms have also been documented to persist, despite clinical cure of the primary infection (153). While the mechanisms behind this persistence are not yet defined, evasion mechanisms appear to be achieved through parasite-induced neutralization of complement cascades, suppression of macrophage function and inhibition of CD4+ T cell expansion (150, 153). Reactivation can occur with immunosuppression.

**Clinical presentation**

**Asymptomatic infection**

Among regions endemic for *Leishmania*, 30% to 50% of otherwise healthy individuals are asymptotically infected and, unless immunity is suppressed, will not progress to overt disease (145, 154). How to manage such individuals is not clear, though a number of aspects should be considered, including risk of disease progression if species is associated with VL and the ability to transmit anthroponotic *Leishmania* spp., which has a significant implication for *Leishmania* control efforts. Asymptomatic infections with *L. infantum* have also been documented in HIV-positive individuals; however, as HIV viral loads increase, the risk for developing active leishmaniasis increases dramatically (155).

**Cutaneous leishmaniasis**

While the classic CL lesion is described as ulcerative with raised boarders (Fig. 10A), the morphology of these

---

**FIGURE 10** (A) Characteristic ulcer of cutaneous leishmaniasis with proximal lymphatic spread (courtesy of Dr. Martins Castro and Dr. Lucille K. Georg, CDC PHIL). (B) Air-dried impression smear from the edge of a cutaneous ulcer shows a macrophage containing numerous amastigotes (arrowhead denotes the macrophage nucleus; Giemsa, 1000×). A nucleus and rod-like kinetoplast can be seen within individual amastigotes (arrows).
lesions can vary greatly and alternatively present as nodules, papules or plaques (97). The lesions may be painful or entirely subclinical and can develop over the course of a week to months. Secondary bacterial infections are also of concern and should be considered in individuals whose symptoms persist, despite antileishmanial treatment. CL in immunosuppressed individuals, typically HIV-positive patients, presents much more acutely with larger, more diffuse lesions which enlarge rapidly without proper treatment (156).

**Visceral leishmaniasis**

Visceral leishmaniasis, also referred to as kala-azar, black fever, or Dumdum fever, is considered to be among the most severe forms of leishmaniasis. Regardless of whether an individual is immunosuppressed, primary infection or reactivation of a latent infection typically presents with spiking fevers that subsequently become continuous and with hepatosplenomegaly, pancytopenia, anemia, anorexia and weight loss. VL has also been associated with stimulation of B cells leading to polyclonal hypergamaglobulinemia, which may be misdiagnosed as multiple myeloma (145). The incubation period can last anywhere from 10 days to one year (147). In regions where infection is endemic, the onset may be more gradual, with vague symptoms and a general feeling of ill health. The onset in immunologically naive patients (e.g., migrants, soldiers, and travelers to areas of endemic infection) may be acute, with high fever, chills, anorexia, malaise, weight loss, and frequently, diarrhea. This syndrome can easily be confused with typhoid fever, malaria, or other febrile illnesses caused by bacteria or viruses.

The majority of AIDS patients present with the classic picture of VL; however, asymptomatic CL, MCL, diffuse cutaneous, and post-kala-azar dermal leishmaniases (usually caused by *L. infantum*) can be seen (146). Cutaneous lesions in VL are being reported increasingly frequently for patients with HIV, and their significance is still somewhat unclear (157–159). Lesions often do not present a uniform or specific appearance and occur as erythematous papules and hypopigmented macules on the dorsa of the hands, feet, and elbows, as small subcutaneous nodules on the thighs, and as scaly plaques on the face.

Among SOT recipients, 77% of leishmaniasis cases have occurred in kidney recipients, which is not surprising as this is the most commonly transplanted organ. The major disease manifestation is VL, followed by MCL and less often CL (160). While this group of patients are at risk for development of leishmaniasis due to primary or reactivated infection, a third route of transmission to be considered is iatrogenic acquisition through transplant of an infected organ or contaminated blood products (147). The median time to development of VL is 17 days for early presentation and 18 months for late presentation (160). VL presents classically in these patients, with fever, hepatosplenomegaly and pancytopenia, and, similar to HIV/AIDS patients with low CD4+ T-cell counts (<200 cells/ml), relapse following adequate treatment is common. Unfortunately, as VL may mimic other more common infections (e.g., disseminated mycobacterial infections, histoplasmosis, lymphoma, etc.), the diagnosis is often delayed or missed entirely, which for transplant recipients can lead to graft dysfunction and loss (147, 161).

**Diagnosis**

In many endemic regions, diagnosis of leishmaniasis is often made based on clinical findings and regional epidemiologic factors. However, confirmation of the clinical diagnosis and identification of the infection in nonendemic regions can be achieved by visualization of the amastigotes in clinical specimens using microscopy or culture, demonstration of parasite DNA by PCR or by detection of *Leishmania* antigen. While any of these methods can be applied to either immunocompetent or immunosuppressed individuals, some caveats exist and are discussed below.

**Direct detection by microscopy**

*Leishmania* amastigotes can be detected in different clinical specimens, including buffy coat smears, tissue biopsies (e.g., cutaneous, bone marrow, spleen, lymph node, liver, etc.) and tissue aspirates (e.g., bone marrow, spleen, etc.). Wright’s or Giemsa stains can be used for reliable detection of amastigotes in any of the aforementioned specimen types. Notably, buffy coat preparations show a significant difference in *Leishmania* amastigote detection between samples collected during the day (46%) and those collected during the night (66%), suggesting that this nocturnal periodicity should be considered during specimen collection (162). While flagellated promastigotes are released into the bite site by infected phlebotomine sand flies, they rapidly differentiate to the amastigote form and therefore amastigotes are generally the form seen in clinical specimens. *Leishmania* amastigotes are usually seen within macrophages and are ovoid in shape, measuring 1 to 5 μm in length by 1 to 2 μm in width (Fig. 10B). Importantly, *Leishmania* and *Trypanosoma cruzi* amastigotes are morphologically similar, having similar dimensions and both pos-
sensing a nucleus and kinetoplast. Therefore, correlation with clinical disease and exposure are important in differentiating these two parasites. It is also important to note that the different *Leishmania* species are morphologically identical, and therefore additional methods (e.g., PCR and DNA sequencing) are required for species identification.

While splenic puncture or aspiration yields the highest rate of organism detection (>95%), it carries a high degree of risk for the patient, including death due to splenic laceration, and is therefore not commonly performed in nonendemic setting (35, 143). In lieu of splenic sampling, bone marrow aspirates or biopsies are generally performed. The sensitivity of amastigote detection in bone marrow samples varies depending on the patient population. For SOT recipients, the sensitivity from bone marrow is 98%, compared to a sensitivity of 70% to 81% among HIV-positive patients (145). It is therefore important to prepare multiple smears for examination and to remind providers that while positive findings indicate infection, negative pathology reports do not rule out disease.

**Culture and animal inoculation**

While culture and animal inoculation studies are likewise helpful to confirm a diagnosis of leishmaniasis, these methods are not routinely available in clinical laboratories, require rapid transport of patient samples to the laboratory for inoculation, and can take several weeks to yield a positive result. Therefore, sole reliance on culture or animal inoculation studies is not recommended, especially if alternative strategies are available (e.g., PCR, microscopy, serology). Classically, *Leishmania* culture is performed using Novy-MacNeal-Nicolle agar with sensitivities in HIV-positive and transplant patients ranging from 70 to 82% depending on the specimen submitted (145). Culture may be used to amplify the organisms in a specimen so that species identification using molecular testing or the older isoenzyme enzyme method can be performed. Culture may be provided by the CDC, which provides direct consultative services and kits for specimen collection and transport.

**Molecular methods**

NAATs have excellent sensitivity and specificity for direct detection of *Leishmania* from clinical specimens, for identification of causative species, and for assessment of treatment efficacy (35, 97). Most studies have evaluated the performance of NAATs on blood and bone marrow with sensitivities ranging from 72 to 100% and 82 to 100%, respectively (145). NAATs performed on whole blood or buffy coat preparations have also been recommended as a means to monitor response to antileishmanial therapy and as a noninvasive method to detect relapse infections in endemic regions. *Leishmania* parasite threshold levels have also been identified, which can aid clinicians in predicting onset of symptoms and VL in HIV-positive individuals from endemic regions (160). One such study reported that a parasitemia of 0.9 parasites/ml detected 12 months following completion of therapy could predict relapse of infection with a sensitivity and specificity of 100% and 90.9%, respectively (163). Unfortunately, molecular assays for *Leishmania* are not routinely available in clinical laboratories located outside of endemic areas, and comparison of parasitemia levels across different assays is not recommended. There is an FDA-cleared real-time PCR (SMART Leish) for detection of *Leishmania* species and specific identification of *L. major*, but its use is largely restricted to the U.S. Department of Defense.

**Serology**

Detection of antibodies to *Leishmania* spp. is an alternative and rapid approach for diagnosis of leishmaniasis. Multiple different serologic methods have been used over the years to help diagnose leishmaniasis, specifically VL, including direct agglutination tests, IFAs, ELISAs, and immunochromatographic assays (ICAs). While detailed sensitivity and specificity data will not be presented here, in general, serologic assays appear to be less sensitive among HIV-positive patients, regardless of the method, compared to individuals who are immunosuppressed due to other conditions (145). Briefly, while direct agglutination tests are exceedingly sensitive for detection of *L. donovani* infections (>95%) due to induction of polyclonal B-cell activation and high immunoglobulin levels, development of commercial direct agglutination tests has been stifled due to high levels of cross-reactivity with other trypanosomatid species and the need for large specimen volumes for antigen purification (164). Despite the high sensitivity documented for IFAs, these assays are likewise not routinely adopted, particularly in endemic regions, due to the need for expensive microscopes. While ELISAs are more routinely available for VL, the sensitivity and specificity of these varies greatly (165, 166).

ICAs, specifically those impregnated with recombinant K39 (rK39) have gained popularity for a number of reasons. First, rK39 is an immunodominant 39-amino acid protein related to the kinesin family of proteins, and it is conserved between *L. donovani, L. infantum* and *L. chagasi* (the latter two species are causative agents of
VL in Latin America) and predominates in amastigotes (167). Among immunocompetent individuals, the sensitivity of the rK39 ICA for detection of VL is greater than 90%, regardless of the causative agent (97, 147). Importantly, rK39 antigen is not found in or expressed at high levels in Leishmania spp. causing either CL or MCL, and therefore this assay should not be relied upon to diagnose these disease manifestations (168).

Secondly, the rK39 ICA is an attractive assay as it can be performed rapidly (10 minutes) and can be used in resource-limited settings. Three key limitations for the rK39 ICA and other serologic assays are 1) antibodies may be detected in otherwise asymptomatic patients; and 3) immunosuppressed patients may be falsely negative by serologic assays, despite active disease and high-level parasitemia (164, 169). Therefore, leishmaniasis should not be ruled out solely based on a negative serologic assay in this patient population.

Treatment
Alongside directed therapy, most patients will require supportive care, especially if they are malnourished or have other infections (e.g., tuberculosis, HIV, pneumonia.) Historically, pentavalent antimony compounds, such as sodium stibogluconate, were considered the drug of choice for treatment of VL. Due to the high toxicity of these agents, increasing treatment failures and resistance, particularly among HIV-positive patients, these compounds are no longer recommended as first-line treatment for VL (35). Recently, the WHO and other government agencies, including the FDA, CDC and American Society of Transplantation, unanimously recommend liposomal amphotericin B as the preferred treatment regimen for both HIV-positive patients and those having undergone organ transplantation (141, 170–172). Repeat VL episodes in HIV patients are of significant concern as relapse infections become increasingly difficult to treat and are often associated with consistently low CD4+ T-cell counts. European studies suggest that following effective treatment of the primary infection and institution of HAART, secondary prophylaxis be considered. While data on secondary prophylaxis are limited, use of amphotericin B reduced relapse rates from 80% to 50% in the year following primary infection (173). Finally, while miltefosine is an alternative agent active against VL, experience with this drug is limited, though from the available data, efficacy appears sufficient with acceptable levels of toxicity (145). Regarding treatment of CL or MCL, the appropriate regimen varies depending on the species, and therefore species identification, typically performed by NAAT, is required for selection of the correct therapy (145).

Control
At the time of this publication, there are no vaccines available or in development against Leishmania spp., and therefore control of both the vector and reservoir hosts are key aspects of most public health control programs in endemic regions. Despite the increasing number of Leishmania infections that occur among transplant recipients, donor screening for Leishmania is not currently recommended by any guidelines. While some studies suggest recipient screening for the presence of Leishmania antibodies prior to immunosuppression, particularly for patients in endemic areas, the clinical utility of this has not been fully elucidated (145). Furthermore, a Brazilian study found that among liver-transplant patients who were positive for the presence of Leishmania DNA prior to transplant, none developed VL during the 2-year follow-up, despite the absence of preemptive prophylaxis (174). Immunocompromised patients should be advised to use appropriate insect repellents when exposed to endemic areas.

Trypanosoma cruzi (Chagas Disease)
Introduction
American trypanosomiasis, or Chagas disease, is a zoonosis caused by Trypanosoma cruzi, a protozoan parasite transmitted by hematophagous triatomine insects (kissing bugs, reduviid bugs) (175). Similar to Leishmania, Trypanosoma species are members of the order Kinetoplastida, due to the presence of a kinetoplast. Within the Trypanosoma genus are over 25 species, of which three have been identified in humans: T. cruzi, T. rangeli, and T. brucei. Among these three, T. rangeli is not considered pathogenic to humans; however, its geographic distribution overlaps with that of T. cruzi, and chance detection of T. rangeli either through serology or histopathology can lead to misdiagnosis of Chagas disease (176). T. brucei, found throughout Africa and transmitted through the bite of a tsetse fly, is the causative agent of African sleeping sickness, and while it leads to significant morbidity and mortality, it is not considered an opportunistic infection of immunocompromised hosts; therefore, it will not be discussed further here.
Epidemiology
Historically, *T. cruzi* infections were localized to Latin America, particularly in regions with poor housing conditions favored by triatomid bugs. With increased migration of infected individuals from rural areas to urban cities both within and outside of Latin America, the distribution of this organism has spread and it has now been detected as far north as Arizona and Texas (177). Approximately 8 to 10 million individuals in Central and Latin America and the Caribbean are infected with *T. cruzi*, while 300,000 individuals are estimated to be infected in the United States (177, 178). Most infected individuals in the U.S. acquired their infection in endemic regions, although rare infections have been reported in individuals who have not travelled outside of the United States (49). In 2007, screening of blood donations began in the United States and between 2007 and 2012, over 1600 individuals with antibodies to *T. cruzi* were identified, with the highest seroprevalence rates occurring in California and Florida (177).

Life cycle and transmission to humans
Triatomine insects are the exclusive insect vectors for *T. cruzi*. Over 130 triatomine species have been identified and each can theoretically support *T. cruzi* development; however only five (*T. infestans, T. dimidiata, T. brasiliensis, R. prolixus, and P. megistus*) prefer living in human habitats and are of epidemiologic importance in Latin America. Triatomine insects are also endemic to the U.S. Eleven species have been identified in the U.S. to date, and *T. cruzi* DNA has been detected in all but two species; however, autochthonous transmission is thought to only rarely occur in the U.S. due to differences in vector behavior and biology (175). During a blood meal, triatomine insects defecate near the bite site, and due to the intense itching caused by triatomine saliva, individuals unknowingly rub the feces containing the infectious *T. cruzi* trypomastigotes into the bite wound, eye or nearby mucosal surfaces (179). The flagellated trypomastigotes invade various cells immediately inside the wound and transform into amastigotes. Intracellular amastigotes divide rapidly by binary fission, filling the cell and transforming back to trypomastigotes, rupturing the host cell in the process. Trypomastigotes disseminate through the bloodstream and can infect almost any tissue, repeating the amastigote-trypomastigote cycle. Triatomine insects may become infected during ingestion of a blood meal containing circulating trypomastigotes, thus continuing the parasite life cycle.

*T. cruzi* can also be transmitted through other routes, including vertically to the fetus, by blood transfusion and organ transplantation (180). In endemic areas, approximately 1% to 10% of infants are born with congenital Chagas disease to *T. cruzi*-infected mothers. The first documented case of *T. cruzi* transmission by blood transfusion occurred in 1952; however, with implementation of serologic screening of blood products in 2007 in the United States, the risk of transfusion-associated infections is now quite low. Receipt of an infected donor organ may also be a significant risk factor for development of Chagas disease in the organ recipient; only 19% (3 out of 16) recipients of *T. cruzi* infected kidneys in one study went on to develop infection (181), indicating that this mode of transmission is not universally effective. Infection can also be acquired through ingestion of fruit or sugar-cane juice contaminated with infected triatomine insects or their feces (175). Transmission through vector-borne routes accounts for 80% of all infections, with the remaining 20% thought to occur through blood transfusions or organ transplantation due to increasing urbanization.

Pathophysiology
Macrophages are the primary defense against *T. cruzi* infections, destroying infectious trypomastigotes in the phagolysosome. However, some trypomastigotes survive, invade the cytoplasm and differentiate into amastigotes to propagate the infection. A strong Th1 cell response is essential for parasite control, and high mortality rates have been described in mice deficient for IFN-γ, inducible nitric oxide synthase and IL-12 (182). Acquired immunity, specifically IgG subclass 2 antibodies to *T. cruzi* trypomastigotes, is likewise important for protection (183). This suggests that an induced immune response to *T. cruzi*, for example through vaccination, may be protective during the acute stage of infection and against re-infection.

Clinical presentation
The clinical syndromes associated with Chagas disease are categorized as either acute or chronic stages of infection, and their presentation can vary depending on how the individual acquired the infection and their immune status.

Acute infection
The incubation period of vector-borne acquisition of *T. cruzi* in an otherwise healthy host ranges from 1 to 2 weeks. Depending on the site of inoculation, two classic signs of acute *T. cruzi* infection may occur (175).
A chagoma, defined as an erythematous, subcutaneous nodule which progressively swells and becomes painful, will form at the bite site and may take 2 to 3 months to subside. If the route of inoculation is through the ocular mucosa, unilateral edema of the eyelids and conjunctivitis occurs, which is collectively referred to as Româña’s sign. The acute stage of infection, characterized by detectable trypomastigotes in stained buffy coat smears, lasts for 8 to 12 weeks (184). While many individuals are asymptomatic during this stage, some may display self-resolving symptoms including fever, fatigue and myalgia. Due to the nonspecific nature of acute *T. cruzi* presentation, many of these individuals do not seek medical attention, thus allowing the infection to potentially progress to a chronic form. More severe forms of acute infection, including myocarditis, pericardial effusion, meningoencephalitis, and death, occur in fewer than 1% of patients and are most often observed in children under the age of 2 years. Orally transmitted *T. cruzi* infections are likewise associated with severe manifestations, with approximately 80% of individuals becoming symptomatic, 60% of those with atypical ECG findings, and a hospitalization rate of 20% (185).

**Chronic infection**

Approximately 8 to 10 weeks after infection, in the absence of effective treatment, individuals will progress to the chronic form of infection. Most patients remain asymptomatic and parasitemia is undetectable in peripheral blood. However, *T. cruzi* can still be transmitted to triatomine insects during this time, and organs or blood harvested from the patient may still be infectious (175). This asymptomatic stage of Chagas disease is termed the indeterminate form of chronic infection and may last for years to decades. Importantly, 20% to 30% of these individuals will progress to evident cardiomyopathy or gastrointestinal (e.g., megacolon, megaesophagus) disease over a 10- to 30-year period (184). *T. cruzi*-associated cardiomyopathy can involve all heart chambers and is due to both parasite persistence in muscle tissues and immune-mediated inflammatory myocardial injury. Early signs of cardiac involvement include abnormal ECG readings, right-bundle branch or left anterior fascicular block and segmental left ventricular wall abnormalities. Without prompt treatment, progression of these manifestations can lead to permanent damage, requiring use of pacemakers to control bradycardia or, in more dire cases, heart transplantation (97). Chronic *T. cruzi* infection leading to gastrointestinal disease is less common and is typically found in patients from the southern Latin American countries.

This geographic variation is likely due to differences in the dominant *T. cruzi* genotype present between the two regions (175). The esophagus and colon are the organs primarily affected, and the associated long-term damage is due to infection and destruction of intramural neurons. Patients will present with odynophagia, weight loss, cough, and if the esophagus or colon are involved, regurgitation or prolonged constipation, bowel ischemia, and megacolon, respectively (184, 186).

*T. cruzi* infections in organ transplant recipients can arise through either receipt of an infected organ or from activation of latent infection (175, 187). Primary infection in a recipient of a *T. cruzi*-infected organ presents in delayed fashion (median time to symptoms 112 days, range 23 to 420 days) and with severe manifestations, including high fever, malaise, anorexia, hepatosplenomegaly, acute myocarditis, and decreased cardiac function (49). For chronically infected individuals undergoing organ transplantation, some experts recommend initiation of preemptive treatment prior to immunosuppresion. However, there is no evidence that pretreatment decreases the risk of future reactivation, and the organ toxicities associated with antitrypanosomal drugs further complicate this issue (188). Therefore, successive monitoring for disease recurrence posttransplant, by NAAT or blood smears, is advised.

The majority of *T. cruzi* reactivation data have emerged from kidney and heart transplant patients. Among kidney transplant recipients, the risk of reactivation is highest during the first year posttransplant. While some individuals may remain asymptomatic, the majority develop subcutaneous involvement, similar in appearance to erythema nodosum, and panniculitis. Without proper treatment, these lesions may ulcerate. Importantly, more severe manifestations of infection, including myocarditis, gastrointestinal involvement and encephalitis, are rare (177, 189). For patients with end-stage Chagas cardiomyopathy, heart transplantation is an acceptable treatment of choice, particularly in Latin America, where this condition is the third leading cause of heart transplants (190). Following transplant, 27 to 43% of individuals develop reactivated disease, which can present similar to kidney transplant patients, though myocarditis is also often observed (177). Importantly, for these individuals, the differentiation of reactivated Chagas disease from that of organ rejection, which often requires heart biopsy and demonstration of *T. cruzi* amastigotes. Similar to other transplant recipients, routine monitoring by NAAT and/or blood smears is necessary to detect reactivation as early as possible.
Approximately 1% to 5% of the HIV-positive population in Latin America is coinfected with *T. cruzi*. Infection in these patients is often a result of reactivated infection which occurs when CD4+ T cell counts fall below 200/μL. Notably, almost 6% of these patients are seronegative for antibodies to *T. cruzi* (189). Chagas disease has now become an AIDS-defining illness in Latin America. Unlike other immunosuppressive conditions, *T. cruzi* infections in HIV/AIDS patients often present as encephalitis or meningoencephalitis with or without myocardial involvement. Because of the immune deficiency associated with HIV, concomitant infection with *T. cruzi* may be difficult to recognize, particularly in individuals who have moved to areas where the disease is not endemic. *T. cruzi* infection can also have a protracted asymptomatic course in HIV patients.

D **iagnosis**

The best-suited method for detection of *T. cruzi* is dependent on the stage of infection (i.e., acute, chronic, reactivated), the status of the patient’s immune system, and assay availability. While direct detection methods such as microscopy and NAATs are best suited for acute forms of the disease, serologic methods are preferred to identify chronically infected individuals.

D **irect detection**

During the acute stage of infection, parasitemia levels are typically high and motile *T. cruzi* trypomastigotes are readily detectable by direct microscopy from either anticoagulated blood or buffy coat preparations. Permanent preparations of these specimen types using hematoxylin and eosin (H&E), Giemsa, or Wright’s stains are also suitable. In humans, the amastigote and trypomastigate *T. cruzi* forms can be detected, and both forms display a prominent kinetoplast (Fig. 11). Trypomastigotes are slender, spindle-shaped and approximately 15 to 24 μm in length by 3 μm in width, with an undulating flagellum which is important for both motility and adhesion and a large posterior kinetoplast. Unlike other *Trypanosoma* spp., *T. cruzi* trypomastigotes often assume characteristic C or U shapes in stained blood smears, although this is not a reliable diagnostic feature. The amastigote form multiplies within virtually any cell, prefering cells of mesenchymal origin, such as reticuloendothelial, myocardial, adipose, and neuralgial cells. *T. cruzi* amastigotes are approximately 1.5 to 5 μm in length and are typically round to oval in shape. As parasitemia dramatically decreases within 90 days of infection, even in the absence of treatment, the sensitivity of microscopy declines significantly over time (97). For HIV-positive patients presenting with neurologic symptoms due to *T. cruzi* infection, direct microscopy in CSF has a sensitivity of 65 to 80% (189).

It is important to note that amastigotes of *Leishmania* species are morphologically similar to those of *T. cruzi*; therefore, correlation with the clinical presentation and additional testing (NAATs, immunoblotting, culture and serology) is recommended. Finally, aspirates from chagomas and enlarged lymph nodes also can be examined for amastigotes and trypomastigotes.

C **ulture**

While culture on Novy-MacNeal-Nicolle media and other media is possible, this technique requires 2 to 4 weeks to visually detect the organism and is not routinely available in clinical laboratories.

M **olecular methods**

Although not routinely available, NAATs targeting a variety of different genes have been applied to detect acute and chronic infection (97, 191). NAATs are particularly useful in cases of chronic Chagas disease, both directly from blood and from tissue biopsies, due to increased sensitivity. However, as NAATs may be positive in the absence of disease reactivation, monitoring for relapse should be done by routine microscopic evaluation of blood smears (192). Alternatively, use of quantitative NAATs to document increasing parasite burden can also be used to diagnose reactivation and appears to be more sensitive than microscopy alone (175). Such assays are primarily available in Latin America and their utility has not yet been studied in the United States.

S **erology**

Serologic methods to detect antibodies to *T. cruzi* are the method of choice for diagnosis of chronic Chagas disease. There are currently a number of anti-*T. cruzi* antibody ELISAs, two of which are FDA-cleared for diagnostic testing in the United States and have sensitivity and specificity ranges of 93 to 100% and 92 to 100%, respectively (193). False-positive results are routinely found in patients infected with malaria, *Leishmania*, and *Toxoplasma*, and nonspecific results have also been observed in individuals with systemic lupus erythematosus and rheumatoid arthritis. Due to these limitations, it is recommended that two serologic tests based on different antigens or techniques be used in parallel to increase accuracy (175, 194). Finally, serologic testing should not be used to evaluate severely immunosuppressed patients as they may be falsely negative, nor should these...
methods be used to monitor patient response to treatment or to document relapse or reactivation.

**Treatment**
Although numerous drugs have been utilized to treat Chagas disease over the decades since it was first discovered, few have proven to be effective. Currently, the drugs of choice are limited to benznidazole and nifurtimox (27, 179). These agents are effective and proven to minimize disease severity, eradicating both the trypomastigote and amastigote forms in 70 to 80% of acutely infected individuals (177). While both agents require prolonged courses, benznidazole is preferred due to fewer toxic side effects. Unfortunately, the efficacy of these agents to treat chronic Chagas disease is limited. Symptoms associated with megaesophagus and megacolon may be treated with dietary measures or may require surgery. Patients with chronic chagasic heart disease may receive supportive

---

**FIGURE 11** (A) *Trypanosoma cruzi* trypomastigotes in a Giemsa-stained peripheral blood film. Note the characteristic large kinetoplast at the posterior end, central nucleus, and free flagellum at the anterior end (top, 1000×). (B) *T. cruzi* amastigotes in cardiac tissue (H&E, 400×, inset 1000×).
therapy or may be managed, in some cases, with pacemakers. Treatment of immunosuppressed individuals does not differ from that of otherwise healthy patients (175).

Control
Human infections occur mainly in rural areas where poor sanitary, socioeconomic and housing conditions provide breeding places for reduviid bugs. Although 12 species of triatomid insects are found within the United States, none are adapted to household habitation. In general, individuals should avoid sleeping in thatch, mud, or adobe houses, and bed nets should be used by persons sleeping in these types of houses. Travelers to endemic areas who stay in hotels, resorts, or other well-constructed housing facilities are not at high risk for contracting Chagas disease.

While a vaccine against T. cruzi is not currently available, efforts to develop one are ongoing (195). Early detection and treatment of infections are recommended to prevent progression to chronic disease but also to limit dissemination of the infection to naive vectors in nonendemic regions.

Finally, in an effort to minimize blood-borne transmission of T. cruzi, as of 2007 all blood donations in the United States are screened for antibodies to T. cruzi using an ELISA, with confirmatory testing of repeatedly positive samples performed by a radioimmune precipitation assay (194).

Plasmodium Species (Malaria)

Introduction
Plasmodium infections are currently uncommon among immunosuppressed individuals in the U.S. However, as the life expectancy of this patient population continues to rise and more individuals travel abroad, the risk of acquiring malaria increases substantially. Additionally, as immigration from Plasmodium-endemic areas escalates, the risk of organ or blood donor-derived infections likewise increases, as blood and organ donors are not currently screened for Plasmodium infections in the United States. Like T. gondii, Plasmodium species are members of the phylum Apicomplexa. The five main species that infect humans are P. falciparum, P. vivax, P. ovale, P. malariae and P. knowlesi (196, 197).

Epidemiology
The epidemiology of malaria is primarily dependent on the female Anopheles mosquito, and more specifically on its density, longevity, biting habits and transmission efficacy. A. gambiae, found throughout Africa, is considered the most efficient malaria vector. It has a relatively long lifespan (2 to 3 weeks), breeds readily, has a tropism for humans and bites within dwellings. An estimated 1.2 billion people remain at high risk for infection with malaria, and in 2013, approximately 198 million cases occurred, of which 584,000 resulted in death (198). Among the five species associated with human disease, P. falciparum causes the greatest morbidity and mortality, followed by P. vivax (199). P. falciparum predominates in Africa, Haiti, the Dominican Republic and New Guinea, while P. vivax is primarily found in the Americas and western Pacific islands. Both species are equally distributed in the Indian subcontinent and eastern Asia (200, 201). Of the more uncommon species, P. malaria and P. ovale are restricted to sub-Saharan Africa, and P. knowlesi infections have thus far been localized to areas of Malaysia, the Philippines, Thailand, and Myanmar (202). Until recently, P. knowlesi was considered infectious only to Old World monkeys; however, over 300 human infections have been reported to date (203).

In many endemic areas, individuals are often infected repeatedly throughout their lifetime and in some regions are inoculated with Plasmodium more than once per day. While mortality is high among children in these areas, those who survive into adulthood develop immunity to malaria and are often asymptomatic during repeat infection (204). In the United States, the vast majority of reported malaria cases are associated with travel to or immigration from endemic areas, and since 1973, the CDC has reported a steadily increasing trend of infections ranging from 1,300 to 1,900 cases annually (205).

Life cycle and transmission to humans
Plasmodium species have a fairly complex life cycle, undergoing both asexual and sexual replication, with multiple different forms found in both humans and mosquito vectors (35, 201). Human infection begins following release of infectious sporozoites from a female Anopheles spp. mosquito while she takes a blood meal. Sporozoites disseminate to the liver, infect hepatocytes and over the next 7 to 16 days, differentiate and replicate into thousands of merozoites, all contained within a intracytoplasmic vesicle referred to as the schizont. The schizont, along with the host hepatocyte, ultimately ruptures, releasing thousands of merozoites into the bloodstream. Some Plasmodium species (i.e., P. vivax and P. ovale) can persist in a dormant phase within hepatocytes and lead to relapse infections months to years following initial infection. Released merozoites infect erythrocytes and undergo asexual replication,
resulting in formation of a schizont and eventual rupture of the erythrocyte, allowing for invasion of new erythrocytes. Depending on the species, this erythrocyte invasion cycle occurs every 1 to 3 days, coinciding with febrile episodes. A small fraction of merozoites form male and female gametocytes, which also circulate in the bloodstream and are the forms that are infectious to the mosquito. Following ingestion by a female Anopheles mosquito, the gametocytes mature and fuse to form a zygote, which eventually develops into an oocyst harboring the human-infectious sporozoite forms. The sporozoites are released and travel to the mosquito salivary glands in preparation for the next blood meal, completing the parasite life cycle. Representative forms of the various life cycle stages are shown in Fig. 12.

The key to the Plasmodium life cycle is the Anopheles mosquito, the only genera of mosquito able to support replication of the parasite. While over 430 species of Anopheles species exist, only 30 to 40 species are competent to transmit Plasmodium and are found on every continent except for Antarctica. Therefore, although malaria is eliminated in many parts of the world, including the United States, there is a potential risk for reintroduction of the disease in all regions where the Anopheles mosquito is found (206). Finally, while vector-borne transmission of malaria is the primary means of infection, Plasmodium can also be spread by blood transfusions, sharing of contaminated needles, organ transplantation and congenitally (207).

Pathophysiology
Malaria pathogenesis involves an interplay of multiple different parasite mechanisms and host genetic factors. Briefly, following infection of red blood cells, Plasmodium spp. digest cellular hemoglobin, producing hemozoin, and also increase RBC membrane rigidity that leads to hemolysis, increased splenic clearance, and anemia. Released hemoglobin and cellular fragments are engulfed by circulating macrophages, stimulating the immune response. Additionally, intracellular Plasmodium spp. produce specific proteins (e.g., PfEMP-1, KAHRP, etc.) which are trafficked to the cell surface and bind to endothelial cell receptors in capillaries and venules. This vascular sequestration of infected cells leads to blood-flow obstruction, breakdown of the endothelial barrier and inflammation (208). Plasmodium infections are also associated with strong CD4+ T-cell activation and a corresponding robust proinflammatory Th1 cytokine response, including to production of TNF-α and IFN-γ.

Interestingly, studies suggest the homozygous polymorphisms in the TNF promoter region are associated with a 7-fold increased risk for severe or cerebral malaria (209). Other host genetic factors associated with resistance to severe malarial infections include alterations in hemoglobin structure (i.e., sickle cell traits and thalassemia). While individuals with these traits in endemic areas are still susceptible to infection, parasitemia levels are substantially lower and morbidity is decreased (210, 211). Elevated immunoglobulin levels appear protective in individuals who have experienced at least five clinical episodes of malaria, suggesting that ongoing Plasmodium antigen stimulation is necessary for persistence of humoral immunity (212). Importantly, stimulation of a strong CD4+ T-cell response by Plasmodium in patients with malaria and HIV coinfection enables rapid spread of HIV among CD4+ cells and consequently high viral loads, which could in turn enhance HIV transmission and disease progression (213).

Clinical presentation
The severity of malaria infections depends on the infecting species and the age and immune status of the host. Generally, malarial infections can be categorized as uncomplicated or complicated. While older children and adults in Plasmodium-endemic areas have partial immunity to infection and are at low risk for developing severe disease, young children, pregnant women, and travelers to such regions are at a much higher risk for clinical complications and death (214). It is therefore critical that malaria be suspected in any traveler or immigrant from malaria-endemic areas who presents with a febrile illness in the United States.

Following infection, sporozoites reach the liver within 2 to 3 hours, and depending on the species, individuals may remain asymptomatic for 10 to 35 days (201). The clinical manifestations of disease begin upon rupture of the merozoites from erythrocytes. Following synchronization of erythrocyte rupture, the onset of febrile episodes stabilizes, occurring every 48 hours in cases of P. falciparum, P. vivax and P. ovale infections and every 72 hours in cases of P. malariae infection. Due to the dormant liver-stage hypnozoites of P. vivax and P. ovale, in the absence of appropriate treatment to eliminate these forms, relapse infections can occur weeks to months following the initial illness.

Initial malarial infections present nonspecifically with fever, tachycardia, headache, chills, nausea, anorexia, and fatigue (215). Jaundice and splenic enlargement are also often noted. Laboratory findings for these individ-
uals include anemia, thrombocytopenia, elevated transaminases and creatinine (201, 215). While each of the five human-infectious species of *Plasmodium* present similarly, certain differentiating features exist. While *P. falciparum* is the most virulent, leading to the highest rates of morbidity and mortality, *P. vivax* has likewise been associated with severe manifestations of disease, including profound anemia, acute respiratory distress syndrome, circulatory collapse and cerebral malaria (216). The mortality rate for severe *P. vivax* infections ranges from 5 to 15%. *P. ovale* infections are uncommon and progress with fairly mild symptoms; few severe cases have been reported. *P. vivax* and *P. ovale* relapse rates vary depending on strain and region of acquisition. In tropical regions, approximately 80 to 100% of individuals will experience a relapse within 30 days of initial infection, while in temperate regions, 30% of individuals will experience relapse disease in 6 to 12 months (217). Individuals with *P. malariae* infections typically have a mild disease course with low-level parasitemia. However, low-grade infection with *P. malariae* can persist for years, and chronic infection has been associated with nephropathy in children and death within 2 to 3 years in the absence of treatment (218). Infection with

---

**FIGURE 12** *Plasmodium vivax* life cycle stages in the human host: early stage trophozoite (top left), late-stage trophozoite (top right), mature schizont (bottom left) and gametocyte (bottom right); (Giemsa, 1000x). Images from reference 312.
P. knowlesi can manifest with severe clinical symptoms, including hypoglycemia, hypotension, and renal and acute respiratory distress syndrome, similar to P. falciparum and P. vivax (219).

Individuals with complicated or severe malaria are unable to take oral medication, cannot sit upright and are otherwise obtunded. Parasitemia levels in these individuals are typically greater than 5%, and the clinical manifestations of disease are largely due to cytoadherence of parasitized erythrocytes causing capillary leakage, infarcts, and organ damage (220). Common clinical findings associated with severe malaria include hypoglycemia, hepatic failure, coagulopathy, intravascular hemolysis, severe anemia, metabolic acidosis, renal impairment, splenic rupture, acute respiratory distress syndrome, and altered consciousness (220, 221).

In individuals with depressed immune function, malarial disease severity is linked to the level of immunosuppression. Due to the epidemiologic overlap of malaria and HIV in Africa, much of present-day data regarding coinfections comes from studies performed in Africa. Not surprisingly, these data indicate that HIV-positive individuals are notably more susceptible to infection with Plasmodium than HIV-seronegative patients (222–224). Furthermore, CD4+ T-cell levels are a critical determinant for disease progression and severity, with <350 cells/μl being a marker for higher risk (225). As discussed above, malarial antigens stimulate CD4+ T-cell production; however, this increase in cellular defense is ultimately lost in HIV-positive patients. This patient population shows increasing HIV viral loads following Plasmodium infections, and some studies suggest that repeat malarial infections are associated with more rapid progression to AIDS (213).

Posttransplant Plasmodium infections have been documented for all Plasmodium species except P. knowlesi and most often occur due to transplantation of an infected organ rather than through transfusion of contaminated blood products or de novo infection. While the majority of such cases occur in malaria-endemic regions, a number of transfusion-related cases have occurred in the U.S. (205). Due to the high number of renal transplants performed, posttransplant malaria infections are most commonly reported among kidney recipients. The disease course in these patients is typically mild, likely due to less intense immunosuppression (177). Conversely, receipt of Plasmodium-infected livers or hearts is uniformly associated with severe disease progression and death, though a notable contributory factor to these outcomes is the use of hepatotoxic antimalarial drugs (177, 226).

Diagnosis

Though each of the five human-infectious Plasmodium species displays some unique clinical characteristics, identification of the species based on patient presentation and travel history alone is not routinely possible. Therefore, the clinical laboratory plays a key role during the clinical course of malaria infections. Not only is it necessary to identify the Plasmodium agent to the species level in order to guide antimalarial treatment, but it is also important to determine both initial and subsequent parasitemia levels to monitor response to treatment and disease progression.

Direct detection by microscopy

Light microscopic examination of peripheral blood smears remains the most commonly utilized method for detection of Plasmodium infections. Preparation of both thick and thin blood smears is recommended by the CDC (http://www.cdc.gov/dpdx/malaria). While Giemsa staining is preferred, Wright’s or Wright-Giemsa stains are also effective. The sensitivity of light microscopy to detect Plasmodium can vary depending on experience of the interpreting technologist, and therefore the CDC recommends repeat thick and thin smears every 12 to 24 hours until a total of three sets are examined before malaria is ruled out. The thick film is the most sensitive method for detecting infection, while the thin film can be used to identify the infecting species and determine the level of parasitemia (227). The level of parasitemia is important for guiding treatment and determining prognosis; it is currently recommended that parasitemia be determined daily for uncomplicated cases of malaria and every 12 hours for the first 3 to 4 days in cases of severe disease (228).

Regardless of the infecting species, four stages of Plasmodium can be found in blood: the ring, trophozoite, schizont and gametocyte forms. Detailed descriptions of these forms can be found on the CDC website (http://www.cdc.gov/dpdx/malaria) (36). Peripheral blood microscopy can also help detect other infections commonly found in malaria-endemic areas, including filariasis and trypanosomiasis. For travelers returning to the United States from malaria-endemic areas, it is important to differentiate P. falciparum from infection with Babesia species, which can appear very similar in blood smears.

Molecular methods

Multiple molecular methods for detection of Plasmodium infections have been developed in recent years, including NAATs targeting different genes, microarray
assays, mass spectrometry-based methods and flow cytometry (229–231). While many of these methods are still in the early stages of development, NAATs, specifically using PCR, are among the most sensitive and specific assays, though testing remains largely limited to reference laboratories and research settings due to cost and assay complexity. NAATs are generally more sensitive than light microscopy, with an estimated limit of detection ranging from 0.2 to 1 parasite/μl depending on the target gene and assay design (231–233). Additional advantages of NAATs are the ability to detect drug resistance and, with some assay designs, to identify mixed infections with higher sensitivity and specificity than using microscopy alone.

Antigen detection
Rapid diagnostic tests (RDTs) to detect circulating malarial antigen in whole blood or serum arose primarily for use in resource-limited settings and are now widely used for diagnosis of malaria in many settings. These assays are based on immunochromatography, are easy to perform and do not require electricity or special shipping or storage conditions. According to the WHO, there are currently over 100 different RDTs available from over 35 different manufacturers (234). The majority of these products are designed to be panspecific, detecting all Plasmodium species by targeting the conserved Plasmodium aldolase or lactate dehydrogenase antigens (231). Some RDTs may further determine whether the infection is specifically due to P. falciparum by including antibodies to the P. falciparum-specific histidine-rich protein II. Species-specific RDTs for detection of P. falciparum, P. vivax, P. ovale, P. malariae and/or P. knowlesi are also available (231, 233). The overall sensitivity and specificity of these assays, particularly for detection of P. falciparum, in both malaria endemic and nonendemic regions is high, ranging from 88 to 99% and 95 to 100%, respectively (236, 237). The performance of RDTs for non-falciparum malaria is lower. RDTs cannot be used to monitor parasitemia levels or response to antimalarial therapy. Therefore, while RDTs are a significant improvement for patient diagnosis and management in rural areas, experts continue to recommend that RDTs be used alongside microscopy for confirmation of species-level identification and for monitoring disease progression.

Serology
Detection of antibodies to the asexual blood forms of Plasmodium has been reported to be a sensitive and specific method for identification of malaria infections, especially if performed by immunofluorescent assays (238). However, as anti-Plasmodium antibodies develop over the course of 2 weeks following infection and remain positive for 6 months or more following parasite clearance, these assays are not suitable for diagnosis of acute infection. Serologic assays for detection of anti-Plasmodium antibodies are best utilized in blood donation centers and for epidemiologic studies.

Treatment
Regardless of patient immune status, antimalarial regimens are identical. However, those with HIV or on immunosuppressive drugs are often at a higher risk for relapse and recrudescence of drug-resistant parasites (233). Treatment regimens vary depending on the infecting species, the prevalence of drug-resistant malaria in the region of acquisition, the clinical status of the patient, pregnancy and drug allergies. For detailed information regarding treatment of malaria, the reader is referred to the CDC guidelines (239). Briefly, for otherwise uncomplicated malaria due to any of the human-infectious Plasmodium species acquired in chloroquine-sensitive regions (e.g., the Middle East, Central America west of the Panama Canal, Haiti, Dominican Republic), the drug of choice in the United States remains chloroquine phosphate or hydroxychloroquine. For patients infected with P. vivax or P. ovale, treatment with primaquine phosphate to destroy the hypnozoite liver stage is also required. Prior to introduction of primaquine, patients should be screened for glucose-6-phosphate dehydrogenase activity, as those with glucose-6-phosphate dehydrogenase deficiency are at high risk for primaquine-associated hemolysis, which may be life threatening. In regions with known chloroquine-resistant Plasmodium, which includes all malaria-endemic regions not mentioned above, the treatment of choice for P. falciparum infections is atovaquone-proguanil, with alternative regimens including artemether-lumefantrine, quinine sulfate with a tetracycline antibiotic or mefloquine. The first-line treatment regimen for chloroquine-resistant P. vivax or P. ovale infections is quinine sulfate with tetracycline, alongside the primaquine. Finally, for severe malaria due to any of the Plasmodium species, first-line treatment includes quinidine gluconate with either doxycycline, tetracycline, or clindamycin (239).

Control
The goal of controlling and ultimately eliminating malaria from endemic regions has been a WHO objective since the late 1980s. Multiple different strategies for control have been implemented, including improving the
availability of antimalarial drugs and personal mosquito-protective equipment (e.g., mosquito repellents, bed nets) to endemic regions (198). Additionally, efforts to better understand Anopheles ecology for vector control purposes are ongoing, as is research to develop an effective anti-Plasmodium vaccine (240, 241). Phase III trials are ongoing in Africa to evaluate the protective effect of RTS-S/AS01, an antigen vaccine based on a repetitive four-amino-acid sequence expressed on the surface of \textit{P. falciparum} circumsporozoites (242). Results are promising, with a 30 to 60% reduction in the incidence of malaria and an overall favorable safety profile. For travelers from nonendemic regions, initiation of an antimalarial prophylaxis regimen, combined with use of personal protective measures, is highly effective at preventing infections. The choice of which prophylactic regimen to take depends on the destination country, immune status of the patient, and reliability of the patient to take the medication as prescribed.

\textbf{Babesia} Species (Babesiosis)

\textbf{Introduction}

Human babesiosis is an emerging protozoan infection found primarily in the U.S. and Europe. \textit{Babesia} species share multiple features in common with \textit{Plasmodium}, including primary transmission by an arthropod vector, a tropism for erythrocytes and similar presenting signs and symptoms. Similar to the \textit{Plasmodium} genera, \textit{Babesia} species are members of the Apicomplexa phylum. Over 100 different \textit{Babesia} species have been identified, but only a few have been associated with human disease. Traditionally, \textit{Babesia} species leading to human babesiosis have been classified into four clades, based on morphology and life cycle characteristics. More recently, as new isolates emerge, both molecular and immunologic findings have been used for categorization, though initial naming schemes are generally based on short acronyms associated with the region where the isolate was first identified (e.g., Washington: WA1; Missouri: MO1) (97). The species of human importance currently include the following: \textit{B. microti} (Clade 1), \textit{B. divergens} (Clade 3), \textit{B. duncani} (Clade 2, including strains previously referred to as WA1, WA2, CA1, CA3, CA4, CA5, CA6), \textit{B. divergens}-like organisms (Clade 3, including strains previously referred to as MO1, KY), and \textit{B. venatorum} (Clade 2, including the previously referred to EU1 strain) (243–247).

\textbf{Epidemiology}

\textit{B. microti} is the most common cause of babesiosis in the U.S. This species is endemic throughout the majority of the northeastern states, associated coastal islands and in the upper midwestern states (248). Other less commonly encountered species in the United States include \textit{B. duncani}, which appears localized to Pacific coast states (e.g., Washington and California), and \textit{B. divergens}-like organisms, which have been isolated from patients in Missouri, Kentucky and Washington (244, 247). In Europe, human babesiosis is primarily due to \textit{B. divergens}, and the majority of cases have been reported from France, England and Ireland (249). Notably, the natural hosts for \textit{B. divergens} are cattle, and many of the documented cases have occurred in individuals in close contact with these animals. \textit{B. venatorum} (previously referred to as the EU1 strain) has also been identified in Europe as the etiologic cause of four human babesiosis cases, primarily in central Europe (250). As serologic assays for \textit{B. divergens} and \textit{B. venatorum} are cross-reactive, seroprevalence for these two species is difficult to address.

\textbf{Life cycle and transmission to humans}

The \textit{Babesia} life cycle involves \textit{Ixodes} species ticks as the definitive host and rodents, primarily the white-footed mouse (\textit{Peromyscus leucopus}), as the intermediate host. Ticks can also transmit \textit{Babesia} to humans; however humans are considered accidental, dead-end hosts as transmission of \textit{Babesia} from infected humans to naive ticks has not been documented. \textit{Ixodes} ticks typically acquire \textit{Babesia} while taking a blood meal from an infected host and transmit \textit{Babesia} sporozoites to their next host during a subsequent blood meal. In humans, these sporozoites infect erythrocytes, undergo asexual reproduction and develop into merozoites which lyse the cell and infect new erythrocytes, propagating the infection. In humans, this cycle of infection and lysis of erythrocytes is responsible for the observed clinical manifestations. Importantly, while the primary route of human infection is zoonotic through the bite of an infected tick, severe infections have also occurred through transfusion of contaminated blood (251).

\textbf{Pathophysiology}

Similar to other intracellular pathogens, CD4+ T-cells and natural killer cells are critical for a strong host response to \textit{Babesia} infection. Production of IFN-γ promotes both macrophage activity and production of a specific antibody response by B cells. Studies indicate that a strong humoral response is essential for parasite clearance in both healthy individuals and those with impaired cellular immunity (252). While in mild cases of babesiosis some elevation of inflammatory cytokines,
including TNF-α and IL-6, is beneficial, in severe cases these inflammatory markers are produced in excess, leading to hypoglycemia, metabolic acidosis, thrombocytopenia and pulmonary and renal complications (253). The spleen is also a critical component of host defense against severe disease by clearing of infected erythrocytes. Asplenic individuals are therefore at significant risk for complications and prolonged parasitemia.

Clinical presentation
The clinical manifestations of babesiosis can range from asymptomatic or mild disease (generally in immunocompetent hosts) to severe and even fatal infections (immunosuppressed or asplenic hosts). Disease severity has also been linked to the infecting species, with \textit{B. divergens} and \textit{B. duncanii} causing more fulminant infections, though the majority of cases described with these organisms have occurred in asplenic individuals who are already at high risk for severe manifestations.

When \textit{Babesia} is acquired through a tick bite, the incubation period can range from one to 6 weeks for \textit{B. microti} or one to 3 weeks for \textit{B. divergens} (254). Following transfusion of contaminated blood products, the median time to symptom onset is 37 days, though some patients have presented much later (range, 11 to 176 days) (255). Symptoms typically develop gradually, with patients reporting general malaise, weakness and fever, which can be high (>38°C), accompanied by chills and sweats. While lymphadenopathy is not usually evident, the spleen and liver may be palpable. Patients may also report headache, myalgia, anorexia, arthralgia and nonproductive cough (248). Common laboratory findings include thrombocytopenia, elevated liver enzymes, and signs of hemolytic anemia, including low hematocrit, low hemoglobin and elevated lactate dehydrogenase. The majority of clinically evident \textit{B. microti} infections, particularly in otherwise healthy hosts, are self-limiting or successfully resolve within 7 to 10 days on directed antibiotic therapy. Asymptomatic parasitemia may persist for weeks to months following recovery and result in disease recrudescence if the patient becomes immunocompromised.

Individuals at risk for severe disease, regardless of the infecting species, include neonates, patients with HIV/AIDS, those on immunosuppressive therapy, organ transplant recipients, those receiving anti-cytokine agents and individuals over the age of 50 due to declining cellular immunity (256, 257). Acute respiratory distress syndrome and disseminated intravascular coagulation are among the most common clinical manifestations of severe babesiosis, though organ failure and splenic rupture have also been reported. Severe \textit{Babesia} infections are associated with a 6 to 9% fatality rate in immunocompetent individuals and up to 21% in immunosuppressed hosts (257). While infection with \textit{B. duncanii} presents similarly to \textit{B. microti}, symptomatic patients with \textit{B. divergens} infections often present with high fever (>41°C), jaundice, hemoglobinuria, headache, intense sweats and lumen and abdominal pain (258). Despite targeted antibiotic therapy, and in some cases exchange transfusion, the fatality rate for \textit{B. divergens} remains high at 40%.

Diagnosis
Prompt diagnosis of \textit{Babesia} infections is essential for positive patient outcomes, particularly when the patient is immunocompromised. As with most other infections, the available diagnostic methods include direct microscopy, molecular methods and serologic testing. The choice between these methods is largely dependent on duration of symptoms and the patient’s immune status. Should a \textit{Babesia} infection be established, particularly in the U.S., it is recommended that providers also assess patients for coinfection with other agents transmitted by \textit{Ixodes} species ticks, including Lyme disease and anaplasmosis.

Direct detection by microscopy
Definitive diagnosis of \textit{Babesia} infections can be established by detection of trophozoite or merozoite forms in Giemsa- or Wright’s-stained peripheral blood smears. These two stages range in size from 1 to 3 μm and are typically oval to pear-shaped (Fig. 13). The infected erythrocytes are similar in size to noninfected cells and can be either singly or multiply infected. \textit{Babesia} ring forms typically have a pale blue cytoplasm with one or two chromatin dots, similar in appearance to \textit{P. falciparum}. Key distinguishing features between \textit{Babesia} and \textit{Plasmodium} include the following: 1) presence of the \textit{Babesia} Maltese cross (rarely encountered); 2) extraerythrocytic \textit{Babesia} forms; 3) absence of brownish, hemozoin pigment in \textit{Babesia}; 4) absence of schizonts or morphologically distinct gametocytes in \textit{Babesia} infections; and 5) variable size and shape of \textit{Babesia} forms (e.g., oval, racket shape, etc.) (259). Similar to \textit{Plasmodium} infections, microscopy is useful to both establish the infection and to monitor disease progression and response to therapy. This method is useful regardless of the patient’s immune status. A notable limitation to microscopy is that the sensitivity of this method may be low in cases of early infection when parasitemia levels are low (<1%) (97). Therefore,
providers are encouraged to submit fresh samples in patients who present with compatible symptoms, prior to ruling out infection.

**Molecular methods**

NAATs to detect *Babesia* DNA are often significantly more sensitive than conventional blood smear examination. The majority of published assays amplify a portion of the 18S rRNA gene and have a limit of detection between 5 and 10 parasites/μl of blood, equivalent to a parasitemia of approximately 0.0001% (260–262). This sensitivity is a distinct advantage of NAATs, particularly in early disease, and NAATs are preferable to microscopy in the setting of acute disease. Currently, the majority of NAATs are strictly qualitative; therefore, microscopy remains essential for determining parasitemia levels. Additionally, as there are no CE-marked or FDA-cleared *Babesia* NAATs available, there may be significant variability in the clinical performance of these assays between laboratories. Other limitations of NAAT testing include the possibility of detecting circulating *Babesia* DNA released from dead parasites in the absence of ongoing disease. Also, depending on the design, not all NAATs may detect the three major *Babesia* species, leading to the possibility of falsely negative results. Adjunct microscopy would be advantageous in these latter two scenarios.

**Serology**

Immunofluorescence assays are the preferred format for detection of antibodies to *Babesia* as they are semi-quantitative and a change in titers can be monitored over time. *Babesia*-specific IgM antibodies appear approximately 10 to 14 days postinfection, making this methodology of little clinical utility during the acute stages of infection (263). Titters ≥1:64 are interpreted as positive by most laboratories, with single-time-point IgM titers of ≥1:64 and/or IgG titers of ≥1:1024 considered indicative of recent infection (264). Serologic assays are best applied as a means to document past exposure and for epidemiologic purposes. Though blood products in the United States are not yet routinely screened for prior infection with *Babesia*, serology-based assays for this purpose are currently in development. The commercially available serologic assays in the U.S. are specific to *B. microti*, and cross-reactivity between *B. microti*, *B. divergens* and *B. duncani* is not observed, leading to the possibility of false-negative results in patients infected with the less commonly encountered species (248). Additionally, in patients with impaired or depleted humoral immunity, negative serologic results should be interpreted with caution and followed up with either microscopy or NAAT. It is important to note that antibody titers can remain positive for 6 to 12 months or longer following resolution of disease; therefore, repeat testing and correlation with clinical symptoms is necessary for accurate interpretation of serologic results.

**Treatment**

Treatment for babesiosis is indicated for symptomatic patients in whom the infection is confirmed by either a positive blood smear or NAAT and in asymptomatic patients in whom *Babesia* has been detected by microscopy or NAAT for over 3 months (265). Per the Infectious Diseases Society of America guidelines, treatment is not indicated for asymptomatic individuals in the first 3 months following initial detection of *Babesia* parasites as the infection may be self-limited. This practice assists...
in minimizing the emergence of antimicrobial resistance in these protozoa.

Two antimicrobial regimens are used to treat babesiosis: clindamycin and quinine or atovaquone and azithromycin. While generally effective, use of clindamycin and quinine has been associated with treatment failures in splenectomized patients, those with HIV and in individuals concurrently receiving corticosteroid treatment (259). Additionally, this combination is not well tolerated and patients may experience diarrhea and severe cinchonism (266). Atovaquone and azithromycin has emerged as an equally effective alternative to clindamycin and quinine following a randomized control trial completed in 2000 (266, 267). The study also showed significantly fewer adverse events and side effects associated with atovaquone–azithromycin as compared to clindamycin and quinine treatment (15% versus 72%, respectively).

Control
While a vaccine against Babesia species is desirable, particularly for high-risk individuals residing in endemic areas, one is not yet available. Therefore, as with all vector-borne infections, the key to infection prevention lies in undertaking personal protective measures, especially if the individual is asplenic or significantly immunosuppressed. Such measures include avoidance of high-risk zones (e.g., walking through thick underbrush or deciduous forests in endemic areas), and if such exposure is inevitable, use of insect repellants (e.g., ≥20% N, N-diethyl-meta-toluamide [DEET]), wearing long-sleeve pants and shirts and careful examination of tick bites. Regarding protection of the blood supply, particularly in endemic areas, the practice of donor questioning regarding past infection or exposure to Babesia has clearly been unsuccessful. Laboratory-based screening assays are currently being developed, and preliminary studies of blood-screening programs in endemic areas appear promising and effective at decreasing the number of transfusion-transmitted babesiosis cases in neonates (248).

INTESTINAL AND TISSUE HELMINTHS
Of the various helminths that can infect humans, Strongyloides stercoralis has the most well-recognized link to immunosuppression. If detected early, and prior to initiation of immunosuppressive regimens, the infection is easily treatable with oral anthelmintic agents, and the severe morbidity and mortality associated with Strongyloides hyperinfection syndrome can be avoided.

Strongyloides stercoralis (Strongyloidiasis)
Introduction
Strongyloides stercoralis, the causative agent of strongyloidiasis, remains a common agent of infection in tropical and subtropical regions but is also encountered in the United States and can lead to severe, disseminated disease, particularly among immunosuppressed individuals.

Strongyloides species are nematodes, also referred to as “threadworms,” belonging to the order Rhabditida. While over 50 different Strongyloides species have been identified, the majority do not infect humans. S. stercoralis remains the type species and the most common agent of strongyloidiasis worldwide, though human infections with S. fuelleborni, sporadically found in Africa and Papua New Guinea, and with S. myopati and S. procyonis have also been recorded (268, 269).

Epidemiology
Current estimates suggest that 30 to 100 million individuals worldwide are infected with S. stercoralis (269). Strongyloidiasis is considered a neglected tropical disease and remains endemic in many tropical and subtropical regions, including throughout Southeast Asia, Latin America, sub-Saharan Africa and many Caribbean islands. Within these regions, the prevalence of strongyloidiasis can be as high as 50%. Endemic foci also exist in the U.S., specifically in rural areas throughout the southeastern states and Appalachia (e.g., Kentucky, Tennessee, West Virginia) (270). Using stool examination techniques, the prevalence of S. stercoralis in the U.S., including both immigrant and native populations, ranges from 0.4% to 46% (269, 271). The incidence of strongyloidiasis appears to be increasing in both endemic and nonendemic regions and is primarily associated with a decline in sanitary conditions, availability of potable water, and personal hygiene.

Life cycle and transmission to humans
The Strongyloides life cycle is among the most complex of the nematodes; it involves both a free-living and parasitic stage but can also develop an autoinfectious cycle within the host (269, 272). Briefly, rhabditiform larvae released into the environment via human stool will molt and develop into free-living male and female adult worms provided the environment is conducive (i.e., moist soil, warm to temperate conditions, etc.). The adult worms mate and the female produces fertilized eggs from which rhabditiform larvae will hatch and
develop into either the infectious filariform larvae or mature adults, continuing the free-living phase and soil infestation. Filariform larvae penetrate exposed human skin and migrate via the lymphatic system into the lungs and penetrate through the alveoli. The larvae next travel up the bronchial tree, and once in the pharynx, they are swallowed and reach the small intestine where they molt into adult female worms. Adult females thread into the intestinal villi and can survive for up to 5 years without antiparasitic treatment. The females produce fertilized eggs via parthenogenesis, and the eggs hatch to release noninfective rhabditiform. Although most of these rhabditiform larvae are shed via stool into the environment, some can also develop into the infectious filariform phase in the small intestine and subsequently penetrate the small intestine or skin to cause autoinfection. These filariform larvae will either follow the route described above and return to the small intestine, or they may disseminate widely throughout the body. While largely suppressed by an intact immune system, low-level autoinfection can persist undetected and without clinical manifestations for decades in otherwise healthy individuals (273, 274). However, upon immunosuppression, autoinfection can lead to hyperinfection syndrome (HIS) and/or disseminated strongyloidiasis, which can be fatal.

Pathophysiology
The precise host immune response to Strongyloides infection continues to be explored; however, studies clearly indicate an essential role of both B lymphocytes and eosinophils for the development of protective immunity and resistance to severe infection. Briefly, individuals with severe strongyloidiasis or hyperinfection syndrome have significantly lower total IgM and IgG antibody levels compared to individuals with asymptomatic or mild infections (275). Helminth infections also stimulate IgE production and, through stimulation of a Th2 response, release of IL-5, which is associated with activation and proliferation of eosinophils. As these macroscopic nematodes cannot be ingested by macrophages, IgE antibodies will coat the organism and recruit eosinophils which degranulate and release compounds able to degrade the parasite surface, ultimately leading to parasite death. Notably, in individuals with HTLV-1 infection, the Th2 response is dampened, leading to decreased production of both IL-5 and IgE and higher risk for strongyloidiasis (275). While clearly a risk factor for severe strongyloidiasis, precisely how HIV and glucocorticoid use increase this risk continues to be elucidated (275).

Clinical presentation
In otherwise healthy individuals, infection with S. stercoralis may be entirely asymptomatic or may present with intermittent gastrointestinal (e.g., diarrhea, abdominal pain) or pulmonary symptoms (e.g., wheezing, cough, throat irritation) (276). Initial penetration of the filariform larvae often results in a cutaneous reaction, referred to as “ground itch,” at the infection site. Some individuals may also develop recurrent pneumonitis resembling bacterial pneumonia.

Upon immunosuppression, new or chronic S. stercoralis infections can produce severe clinical manifestations including HIS and/or disseminated disease. At greatest risk are patients with impaired cellular immunity due to either genetic factors, malignancy, SOT or SCT, or corticosteroid or cytotoxic drug use. Notably, even short courses of corticosteroids (6 to 17 days) have led to HIS in chronically infected individuals (277). It is therefore critical that all at-risk patients be screened for S. stercoralis infection prior to initiation of immunosuppressive regimens. Additional risk factors for severe strongyloidiasis are infection with HTLV-1 and HIV. The prevalence of S. stercoralis is notably higher in patients HTLV-1 compared to noncarriers in Japan and elsewhere (278–280). Individuals with HTLV-1 produce high levels of IFN-γ and lower levels of IL-4, IL-5, IL-13 and IgE, which are all important for control of S. stercoralis (281, 282).

Hyperinfection syndrome is characterized by an extremely high worm burden due to uncontrolled intestinal replication and pulmonary migration. Patients with HIS often present with pulmonary symptoms, including pulmonary infiltrates, diffuse alveolar hemorrhage and respiratory failure, which may be fatal if not treated promptly. Disseminated strongyloidiasis is characterized by the presence of S. stercoralis in organs outside of its natural parasitic life cycle, including the liver, kidneys, endocrine glands and central nervous system. A secondary consequence of vast nematode migration out of the intestinal tract is the translocation and seeding of these organs with enteric bacteria. This can lead to abscess formation, bloodstream infection and meningitis if the CNS is invaded. While disseminated strongyloidiasis alongside a secondary bacterial infection can be severe, fatalities are much less common compared to HIS (269, 276).

Diagnosis
The diagnosis of strongyloidiasis requires a high level of clinical suspicion as the clinical manifestations of both acute and chronic disease are largely nonspecific and
clinicians in non-endemic regions may not be as familiar with the risk factors for infection. Both direct and indirect methods to detect *S. stercoralis* infection are available, including stool microscopy, stool culture, and serology. Molecular methods to detect *S. stercoralis* nucleic acid have been developed as well, although these are largely restricted to research settings and will not be discussed in detail here.

**Direct detection by microscopy**

In contrast to the majority of other helminths, *S. stercoralis* larvae rather than eggs are detected in stool specimens. Stool microscopy is notoriously insensitive, however, as larvae are intermittently shed and otherwise healthy individuals with a chronic infection typically have low worm burdens. In general, the sensitivity of a single stool examination to detect *S. stercoralis* ranges from 30% to 50% (283). To increase sensitivity, it is currently recommended that at least seven separate stool samples be submitted for microscopy prior to ruling out infection. For patients with HIS or disseminated infection, *S. stercoralis* filariform larvae can also be detected in respiratory samples (e.g., bronchoalveolar lavage fluid, sputum), pleural fluid, peritoneal fluid and CSF (284, 285). Notably, CSF examination reveals elevated protein, decreased glucose levels and pleocytosis with a neutrophilic predominance (269). Direct smear examinations of these samples or staining with Lugol’s iodine is sufficient to detect the larvae. *S. stercoralis* larvae can be seen in intestinal biopsies (Fig. 14).

Given the poor sensitivity of conventional stool parasitic exam, several stool concentration techniques, including the Baermann funnel technique, the Harada-Mori filter paper technique, and the agar plate culture method have been developed (286). Of these, the agar plate method provides the greatest sensitivity and greatest ease of use. With this method, stool samples are plated onto a nutrient agar that can support bacterial growth. If *Strongyloides* larvae are present in the sample, their migration into the surrounding agar will carry fecal bacteria, which then grow to produce visible tracks. Motile larvae can be observed at the end of these tracks. Importantly, samples which are generally considered sterile (i.e., bronchoalveolar lavage fluid, peritoneal fluid) require bacterial supplementation for these tracks to be visible (269, 285). Should culture be undertaken, technologists should be extremely cautious to prevent laboratory-acquired *S. stercoralis* infections as the motile filariform larvae are infectious and can penetrate intact skin. *S. stercoralis* larvae can also be detected on routine bacterial stool cultures. Therefore, any culture plates which exhibit serpiginous bacterial growth that may represent larval tracks should be handled with extreme care.

**Molecular methods**

Multiple different NAATs targeting various genes (e.g., cytochrome-c oxidase subunit I, 18S rRNA, 28S rRNA) have been described for detection of *S. stercoralis* nucleic acid from stool samples (287, 288). Though these assays remain limited to the research realm, the specificity of these techniques approaches 100%, regardless of target. Compared to culture, these assays likewise show excellent performance (90% to 100% sensitivity). However, in cases of low-burden infection, molecular assays do not appear to perform better than direct microscopy or culture (289).

**Serology**

While direct-detection methods provide definitive diagnosis of *Strongyloides* infection, the need for serial sample submission and the requirement of prompt specimen submission to preserve larval viability may be impractical in certain scenarios. Given these limitations, serologic approaches to detect an immune response to *S. stercoralis* have emerged as an alternative diagnostic tool. Multiple serology-based assays have been developed, each differing in the antigen target used for detection (e.g., crude lysate, purified antigen, recombinant antigen) and the methodology (e.g., immunochromatography, ELISAs or luciferase immunoprecipitation systems) (290–292). There are a number of commercially available serologic assays available which detect

![FIGURE 14 Strongyloides stercoralis larvae invading the intestinal mucosa in a case of hyperinfection (H&E, 1000x).](image-url)
anti-Strongyloides IgG antibodies, and overall, the sensitivity and specificity of these assays in cases of proven strongyloidiasis range from 73% to 100% and 65% to 90%, respectively (289). Serologic assays are notoriously insensitive in immunosuppressed individuals, requiring multiple tests and adjunct evaluation (285, 293). Providers are therefore encouraged to submit serum samples for screening prior to initiating immunosuppressive therapy.

From the perspective of assay specificity, ELISAs based on recombinant Strongyloides antigens show improved performance, but cross-reactivity with other helminths, including infection with Filaria species, Taenia species, and Toxocara have been reported (289, 291). Finally, while not commercially available, the luciferase immunoprecipitation systems assay performed at the National Institutes of Health provides the highest combined sensitivity and specificity (97% and 100%, respectively) for detection of Strongyloides-specific antibodies, and cross-reactivity with other helminths has not been observed (292, 294).

Antigen detection
While assays detecting Strongyloides antigen in stool or serum have also been developed, availability of these assays is largely restricted to research settings. These assays are ELISA-based and in animal studies show excellent sensitivity and specificity, with little cross-reactivity seen among other helminths, including Schistosoma mansoni, Fasciola gigantica and Capillaria philippinensis (289). Further evaluation of these ELISAs and development of rapid immunochromatographic versions of these tests is ongoing.

Treatment
Currently, the treatment of choice for strongyloidiasis is two doses of ivermectin taken on two consecutive days or 2 weeks apart, with a reported efficacy approaching 100% (295). Albendazole regimens are typically longer in duration, 3 to 7 days, and efficacy against Strongyloides is considerable lower, 50 to 80%, compared to ivermectin (296). For immunocompromised individuals with either HIS or disseminated disease, reduction of immunosuppressive therapy is an important aspect of infection control, in addition to anti-helminth therapy. While there is no standardized therapeutic regimen for these patients, a prolonged course of ivermectin (5 to 7 days) is usually pursued. Treatment duration with ivermectin is dependent on clinical improvement and daily examination of stool specimens. Often, treatment is maintained in individuals until stool microscopy is negative for 2 weeks (297). However, as direct microscopy lacks sensitivity in cases of low parasite burden, patients should be monitored closely following discontinuation of therapy, and if symptoms recur, the possibility that initial therapy was not curative should be considered. Additionally, failure to respond to treatment on multiple occasions should raise the suspicion of HTLV-1 infection. Finally, prior to initiation of immunosuppression by either corticosteroid use or organ transplantation, individuals who are serologically positive for antibodies to Strongyloides and who have an appropriate exposure history require a preemptive, prophylactic anti-Strongyloides treatment regimen with ivermectin to minimize the risk of hyperinfection following suppression of the immune system (298).

Control
Control of strongyloidiasis is directly linked to improvement in sanitary conditions and stopping the Strongyloides life cycle at the point of rhabditiform filaria release into the environment.

ARTHROPODS
Sarcoptes scabiei (Scabies)
Introduction
Scabies, from the Latin scabere meaning “to scratch,” is a highly contagious infestation with the mite Sarcoptes scabiei and is also referred to as the “seven year itch.” Outbreaks in hospitals and institutions, particularly those for the elderly or mentally handicapped, have been documented worldwide (299). Scabies infections are particularly severe among immunosuppressed individuals, who may ultimately harbor thousands of mites on the skin and develop a form of disease called “crusted scabies” (also referred to as Norwegian scabies). S. scabiei are ectoparasites found within the order Astigmata, which contains the fairly slow-moving, “biting” mites (300). There are currently seven varieties of S. scabiei; however, human infection with non-S. scabiei var. hominis mite is uncommon as these mites do not survive more than a few days on human skin (301).

Epidemiology
Scabies is among the three most commonly encountered skin infections worldwide, following pyoderma and tinea, with an estimated 100 to 300 million individuals infected each year (299, 302). Currently, S. scabiei is considered endemic in many tropical and subtropical areas, including Africa, Central and South America,
India, and Southeast Asia (300). Susceptibility to scabies is not affected by age, gender, race, sex or socioeconomic factors, though certain groups are more likely to be affected, including the very young and young adults, likely due to lifestyle and close contact with other individuals. In temperate regions, scabies is more commonly observed during the winter months, likely due to increased crowding and better survival in cold climates. Cyclical epidemics have been documented, largely coinciding with war and natural disasters (300).

**Life cycle and transmission to humans**

Similar to other arthropods, *S. scabiei* undergoes four developmental stages: egg, larva, nymph and adult. Transmission to humans or other vertebrate hosts occurs through direct skin-to-skin contact or contact with a highly contaminated fomite (303). Importantly, scabies mites do not survive for more than 1 to 2 days off of a host, thus limiting the risk of transmission through contaminated clothing and other shared objects. The adult female will burrow into the host’s stratum corneum, advancing approximately 2 mm per day, and will deposit two to three eggs daily (299, 301). After an average lifespan of 15 to 30 days, the mite will die at the bottom of the burrow. Eggs will hatch 3 to 4 days following deposition, and the emerged six-legged larva will molt up to four times prior to leaving the burrow. On the skin surface, the adults will copulate and the cycle will repeat.

**Pathophysiology**

The intense pruritus associated with scabies is a direct result of a delayed type-IV hypersensitivity reaction in response to the mite, the eggs, the proteolytic burrowing enzymes and mite feces released in the burrows (304). Currently, the exact antigen or antigens responsible for these reactions remain unknown. Infected individuals also develop an antibody response, with elevated IgE and IgG levels, which may be responsible for the rapid host response to reinfestation. A strong cell-mediated immune response to antigens released by the mites during feeding is necessary to prevent severe infestation, and therefore individuals with decreased or altered T-cell function are at much higher risk for progression to crusted scabies (305). Briefly, these predisposing conditions include infections with HIV, HTLV-1, T-cell lymphoma and leukemia.

**Clinical presentation**

Following primary infection, pruritus manifests in approximately 3 to 6 weeks, with more intense itching commonly reported at night. Notably, the incubation period is much shorter, in the range of one to 3 days, in individuals who have been infected previously, likely due to prior priming of the patient’s immune system (299, 300). The resultant lesions are often nondescript, however an associate burrow, if identified, is pathognomonic for scabies. Burrows typically appear as thin red-brown lines, 2 to 15 mm in length. Classically, scabies infections occur in the webs of fingers, the flexor aspects of the wrists, on the exterior of the elbows, the buttocks, periumbilical skin, the ankles and periareolar region in females. The back, palms, soles and face are usually spared in adults. Depending on the extent of infection and the severity of the immune response, which is directly tied to an individual’s immune status, the clinical appearance of the infestation can vary. Due to the intense itching and compromised skin barrier, secondary infections with common skin flora, including *Staphylococcus aureus* or group A *Streptococcus* may occur, leading to cellulitis and more invasive, disseminated infections (306).

Crusted scabies, also referred to as Norwegian scabies, typically occurs in individuals with preexisting, immunosuppressive conditions. Importantly, there is no difference between the *S. scabiei* mites that lead to mild or crusted scabies, and mild cases of scabies do not progress to crusted scabies in otherwise healthy individuals. Crusted scabies is a severe infection characterized by the presence of millions of mites and eggs, unlike in mild cases where the numbers of mites range from 10 to 15. Due to such high numbers of mites, patients with crusted scabies are referred to as “core transmitters” and the source of infections following intervention programs (300). Patients at increased risk of developing crusted scabies include those with leprosy, human T-cell leukemia virus type 1 (HTLV-1), HIV/AIDS and any individual on significant immunosuppressive therapy (307, 308). Crusted scabies initially presents with poorly defined erythematous patches which can occur on any area of the body. Without treatment, these lesions become malodorous, scaly and thick with intermittent crusts and fissures and can overtake the entire integument, become severely debilitating.

**Diagnosis**

A diagnosis of scabies is made on clinical presentation, history and direct observation of the organism from skin scrapings. Key features associated with infection include widespread itching which is more prominent at night, classical distribution patterns and the presence of other
close contacts with similar symptoms. The differential diagnosis includes many other infectious and noninfectious conditions, including eczema, tinea, atopic dermatitis, systemic lupus and seborrheic dermatitis (301).

**Direct detection**

Adult *S. scabiei* range in size from 0.25 to 0.5 mm in length and 0.2 to 0.3 mm in width, and stalked pulvilli, or suckers, are present on the first two legs. These suckers, alongside spur-like claws and spin-like projects on their dorsal surfaces, enable the adults to better grip and pull themselves into the burrows (300, 301). *S. scabiei* eggs are approximately 0.15 mm long by 0.1 mm wide, and the fecal pellets, which are yellowish-brown in color, are roughly 30 by 15 μm (Fig. 15).

Scabies ectoparasites can be identified in the laboratory by examination of properly collected skin scrapings from actively infected sites. Briefly, a drop of mineral oil is applied to the skin lesion and a scalpel blade is used to either scrape or gently shave the top layer of skin. The specimen is subsequently observed under the microscope at 10× magnification. Alternatively, dermoscopy, performed with a handheld dermatoscope, may be performed to directly visualize *S. scabiei* mites and eggs on the skin surface. The classic findings on examination are the dark, triangular heads of the mite within the burrow, or the “delta wing” sign (309).

*S. scabiei* mites can also be detected on bacterial culture of skin scrapings by the presence of serpiginous tracks (similar to those created by *S. stercoralis*) on the agar surface, representative of the bacteria dragged along as the mite moves. This unusual laboratory manifestation can alert the staff to the possible diagnosis of scabies. Although many microbiology laboratories are aware of these unusual findings, personnel can forget to consider scabies in such situations.

**Treatment and prevention**

The first-line therapy for scabies in the United States is topical application of 5% permethrin and oral ivermectin. Importantly, household contacts should also be treated to prevent spread and reinfections (310). Individuals can return to work or school one day following treatment. Additionally, patients should be advised to place potential fomites (clothing, linens, cloth toys, etc.) in a plastic bag for at least 3 days or wash and dry them using high temperatures. Fumigation of living areas is not currently indicated.

---

**REFERENCES**


**FIGURE 15** Sarcoptes scabiei mature mite (left), immature mite (bottom right) and egg (top right) in an unstained preparation of skin scrapings.


Infect Dis with immunosuppression, shortness of breath, and eosinophilia.


